COMMEMORATIVE ISSUE IN HONOR OF CHARLES FRANKLIN CRAIG, 1872–1950

VOLUME 1 January 1952 NUMBER 1

THE AMERICAN JOURNAL OF TROPICAL MEDICINE and HYGIENE

Combining

The American Journal of Tropical Medicine
and The Journal of the National Malaria Society

OFFICIAL ORGAN OF
THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

PUBLISHED BIMONTHLY BY
THE WILLIAMS & WILKINS COMPANY
BALTIMORE 2, U.S.A.

Copyright 1952, By The American Society of Tropical Medicine and Hygiene
Made in U.S.A.

THE AMERICAN JOURNAL OF

Tropical Medicine and Hygiene

Editor-in-Chief, L. W. HACKETT
School of Public Health, University of California
Berkeley 4, California

Editor, Tropical Medicine and Hygiene News
LEON JACOBS, National Institutes of Health
Bethesda 14, Maryland

Editorial Board

G. ROBERT COATNEY, *Chairman*
ERNEST CARROLL FAUST
HAROLD FARNSWORTH GRAY
WILLIAM McD. HAMMON
HERBERT G. JOHNSTONE
EDWIN H. LENNETTE
HARRY MOST
LLOYD E. ROZEBOOM

OFFICERS OF THE
American Society of Tropical Medicine and Hygiene

President
MARTIN D. YOUNG

President-Elect
WILLIAM A. SODEMAN

Vice-President
H. W. VAN HOVENBERG

Secretary-Treasurer
QUENTIN M. GEIMAN
Department of Tropical Public Health
Harvard School of Public Health,
25 Shattuck Street,
Boston 15, Massachusetts

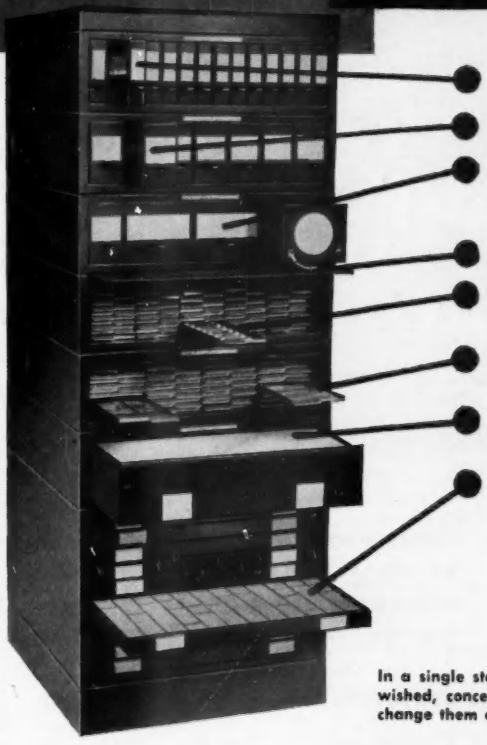
Councilors:	*Term Expires*
GEORGE H. BRADLEY	1952
HARRY MOST	1952
JUSTIN M. ANDREWS	1953
DONALD L. AUGUSTINE	1953
TOM F. WHAYNE	1954
HERBERT G. JOHNSTONE	1954
EDWIN H. LENNETTE	1955
G. ROBERT COATNEY	1955

Editor
L. W. HACKETT

"Individuals interested in any phase of tropical medicine and hygiene, including all arthropod-borne diseases, medical care, and human welfare are eligible for membership. Dues are $8 a year and include subscription to the American Journal of Tropical Medicine and Hygiene. An application blank is bound into this issue of the Journal for the use of interested individuals. Before being submitted to the secretary, each application must be endorsed by two active members of the Society, and a check for $8 (U. S. banks preferred) must accompany the application.

Entered as second class matter March 16, 1921, at the post office at Baltimore, Md., under the act of March 3, 1879.
Subscription price, $10.00 per volume. Single numbers, $2.00.

answer to every laboratory [illegible] need



*1" MICROSLIDE DRAWERS
(for spaced or storage filing)

*2" SLIDE OR TRANSPARENCY DRAWERS
(for spaced or storage filing)

*4" DRAWERS
(for 3¼" x 4¼" slides or index cards. Technilume drawer for built-in illumination optional)

SHELF
(for resting trays or drawers in use)

FLAT-FILING CABINET FOR MICROSLIDES
(slides lie flat in trays; dry quickly; are visible at a glance)

FLAT-FILING UNIT FOR TRANSPARENCIES
(2" x 2" or 3¼" x 4¼" for filing and instant viewing)

ILLUMINATION UNIT
(used in conjunction with Transparency Flat-Filing for viewing)

PARAFFIN BLOCK FILE
(shallow drawers, with vari-sized knock-down cardboard containers)

*Single cabinets can comprise variable assemblies of any of these drawers, thanks to the Technicon Lab-aid unit track design.

In a single stack of Technicon Lab-aid cabinets you could, if you wished, concentrate all these various facilities. Or add to them, change them about, even interchange the drawers in a single unit.

technicon *Lab-aid* filing system

engineered for efficiency . . . . . organized for economy

Every cubic inch of a Technicon Lab-aid filing unit is a live filing inch, without waste space for struts, dividers and the like. For instance, a single unit of 1" microslide drawers, used for close-packed storage filing, will accommodate approximately 6500 slides . . . a gain of up to 45% in capacity.

You can use a compact Lab-aid cabinet on your desk (it's only 19" square) or stack them to any height. Staunch welded-steel construction includes interlocking angles for stability and weight-supporting strength.

THE TECHNICON COMPANY — 215 EAST 149th ST., NEW YORK 51, N. Y.

"prepare for your widow's remarriage"

So heavy was the death toll from malaria in ancient times, that a Chinese manuscript of the third century B. C. advised the traveler preparing for a journey to regions where chang-chi ("malicious air") prevailed to make arrangements for the remarriage of his widow.[1]

Malaria still presents an outstanding medical problem, although great strides have been made in malaria control and therapy. Aralen signalizes an important advance in the treatment of malaria.

ACCEPTED COUNCIL ON PHARMACY AND CHEMISTRY AMERICAN MEDICAL ASSOCIATION

ARALEN® DIPHOSPHATE
Brand of Chloroquine Diphosphate

Available in tablets of 0.25 Gm., tubes of 10 and bottles of 100.

Four Dose Treatment: Adults, 4 tablets, followed by 2 tablets after 6 to 8 hours, and 2 tablets on each of 2 consecutive days. Total only 10 tablets in 3 days.

For Suppression: Adults, only 2 tablets once a week.

Winthrop-Stearns INC.
NEW YORK 13, N. Y. WINDSOR, ONT.

Aralen, trademark reg. U. S. & Canada

....possesses these qualities:

- highly effective—producing rapid symptomatic relief
- few relapses and long periods between relapses
- low incidence of gastro-intestinal irritation
- no discoloration of the skin
- well tolerated in therapeutic doses

AUTHORITATIVELY RECOGNIZED

Extensive investigations carried out under the auspices of the Board for the Coordination of Malarial Studies of the National Research Council established the great value of Aralen diphosphate.[2]

1. Beckman, H.: Treatment in General Practice. Philadelphia, W. B. Saunders Co., 1946, p. 103.
2. Loeb, R. F. (Chairman, Board for Coordination of Malarial Studies) et al: Activity of a New Antimalarial Agent, Chloroquine (SN7618), *J.A.M.A.*, 130:1069, Apr. 20, 1946.

THE AMERICAN JOURNAL OF

TROPICAL MEDICINE & HYGIENE

Vol. 1 *January, 1952* *No. 1*

CONTENTS

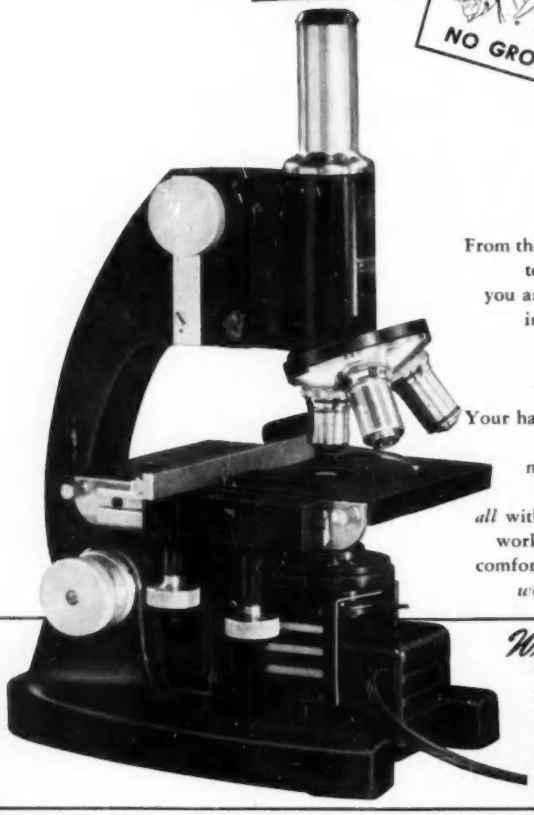
Announcing a new journal. THE EDITOR 1
Foreword. THE COMMITTEE ON THE CHARLES FRANKLIN CRAIG MEMORIAL ISSUE. 2
Portrait, Charles Franklin Craig 4
Charles Franklin Craig: fifty years of work and service in tropical medicine. C. C. BASS 5
Colonel Charles Franklin Craig, M.C., U.S. Army. A salute to the chief. JAMES STEVENS SIMMONS 20
Tropical Medicine in the Armed Forces. WILLIAM S. STONE 27
Research on dengue during World War II. ALBERT B. SABIN 30
Propagation of dengue virus strains in unweaned mice. GORDON MEIKLEJOHN, BEATRICE ENGLAND, AND EDWIN H. LENNETTE 51
Adaptation of dengue virus to the hamster. GORDON MEIKLEJOHN, BEATRICE ENGLAND, AND EDWIN H. LENNETTE 59
Adaptation of the "New Guinea B" strain of dengue virus to suckling and to adult Swiss mice. A study in viral variation. R. WALTER SCHLESINGER AND JACK W. FRANKEL 66
Endemic yellow fever in Panama and neighboring areas. HERBERT C. CLARK 78
Immunization against scrub typhus: duration of immunity in volunteers following combined living vaccine and chemophrophylaxis. J. E. SMADEL, H. L. LEY, JR., F. H. DIERCKS, P. Y. PATERSON, C. L. WISSEMAN, JR., AND R. TRAUB 87
The influence of the National Malaria Society on malaria eradication. JUSTIN M. ANDREWS 100
The present status of malaria in the world. PAUL F. RUSSELL 111
The treatment of malaria. L. T. COGGESHALL 124
The *in vitro* activity of certain antimalarials against erythrocytic forms of *Plasmodium gallinaceum*. D. JANE TAYLOR, EDWARD S. JOSEPHSON, JOSEPH GREENBERG, AND G. ROBERT COATNEY 132
Modern criteria for the laboratory diagnosis of amebiasis. ERNEST CARROLL FAUST 140
Concepts and misconceptions in amebiasis. JOSEPH S. D'ANTONI 146
The cultural requirements of *Endamoeba coli* and the comparative effects of drying on the cysts of *E. coli* and *E. histolytica*. LUCY V. REARDON, ELIZABETH VERDER, AND CHARLES W. REES 155
Experimental *Endamoeba histolytica* infections in rabbits with reference to chemotherapy. GEORGE W. LUTTERMOSER, W. T. HASKINS, NELL COLEMAN, AND J. R. JUMPER 162
The dysenteries in the Armed Forces. ALBERT V. HARDY, RICHARD P. MASON, AND GERALD A. MARTIN 171
Correction 176

EASY DOES IT!

with BAUSCH & LOMB

Dynoptic

LABORATORY MICROSCOPES

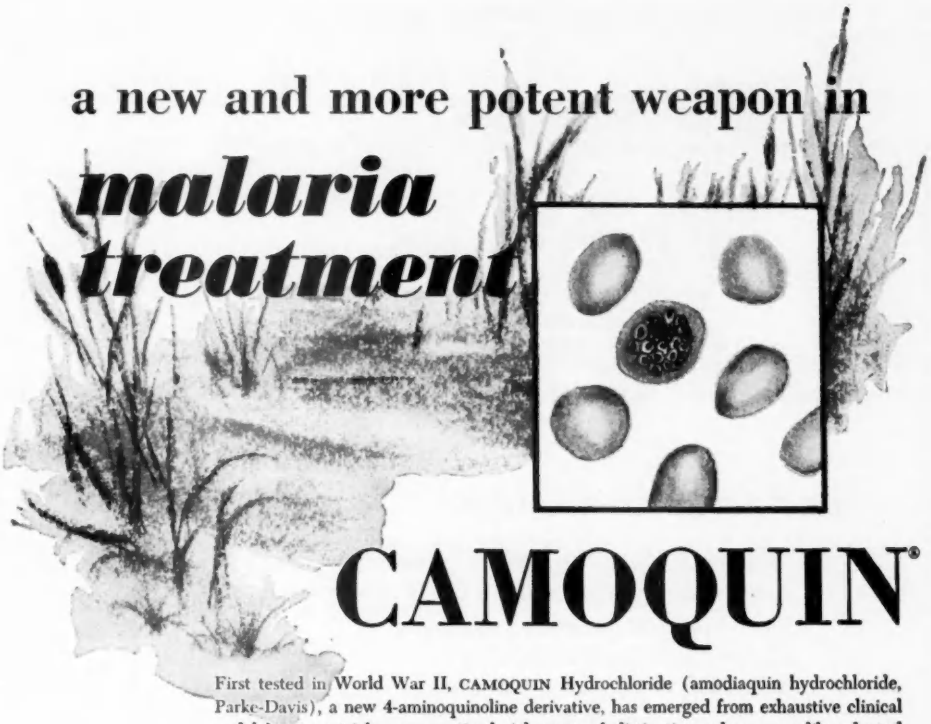


From the moment you position your slide . . . to your final look at the specimen . . . you are enjoying a whole new experience in microscopy. You've never felt such smooth rolling focusing action as Dynoptic ball bearing motion. You've never known such comfort. Your hand is *completely at rest* on the table for operation of fine adjustment, mechanical stage, substage condenser controls, and illuminator switch . . . *all* within effortless reach. You—and your work—will benefit from these *exclusive* comfort and convenience advantages of the *world's finest laboratory microscopes.*

Write for free demonstration and literature. See for yourself how Bausch & Lomb *Dynoptic* Microscopes out-perform any laboratory microscope ever made. Bausch & Lomb Optical Co., 766-13 St. Paul St., Rochester 2, New York.

B&L

a new and more potent weapon in *malaria treatment*



CAMOQUIN®

First tested in World War II, CAMOQUIN Hydrochloride (amodiaquin hydrochloride, Parke-Davis), a new 4-aminoquinoline derivative, has emerged from exhaustive clinical and laboratory trials as an antimalarial agent of distinctive value . . . readily adapted both to therapy and to prophylaxis.

Therapeutically Prompt in Action

CAMOQUIN promptly terminates acute febrile symptoms and signs in all forms of malaria. With adequate dosage, fever is terminated and parasites disappear from the peripheral blood within 48 hours after the first dose. In P. vivax infections, onset of relapse is delayed and in some instances frequency of relapse appears to be materially reduced.

Prophylactically a Dependable Suppressant

Large-scale field experiments have demonstrated conclusively that 0.6 Gm. once every 2 weeks may be relied upon to suppress clinical break-through except in highly virulent infections.

Clinically Well-tolerated

In ordinary therapeutic dosage CAMOQUIN does not give rise to undesirable side reactions. Even in excessive dosage, symptoms of intolerance are mild and transient. Dosage: Therapeutically, 0.6 Gm. as a single dose. (If found necessary, as much as 0.4 Gm. 3 times daily for 5 days may be given.)

Prophylactically, 0.6 Gm. as a single dose once every 2 weeks.

Package Information: CAMOQUIN Hydrochloride as scored tablets containing 0.2 Gm. of the base in folders of three tablets, in boxes of 12 folders (36 tablets), and bottles of 1,000 tablets.

PARKE, DAVIS & COMPANY

MEDICAMENTA VERA · PD CO.

CHEMICALS . . .

You Can Count on

For Better Mosquito Control

THE TRITON COMPOUNDS

This unique group of spreading and emulsifying agents enables you to formulate larvicides that have just the physical properties you require for effective and economical control measures.

RHOTHANE

This insecticide, frequently referred to as TDE or DDD, has generally proved as effective as DDT—frequently superior to it as a larvicide. Fortunately, RHOTHANE is much safer than DDT to aquatic life and this fact has helped entomologists to solve several particularly troublesome gnat and mosquito problems.

Working samples of the TRITONS and RHOTHANE formulations and technical information on these products are yours for the asking.

CHEMICALS FOR INDUSTRY

ROHM & HAAS COMPANY

WASHINGTON SQUARE, PHILADELPHIA 5, PA.

REPRESENTATIVES IN PRINCIPAL FOREIGN COUNTRIES

TRITON *and* RHOTHANE *are trade-marks Reg. U. S. Pat. Off. and in principal foreign countries.*

(In writing to advertisers, please mention the journal—it helps.)

KWELL® LOTION

A SINGLE APPLICATION USUALLY SUFFICES IN

SCABIES AND PEDICULOSIS

The dependable efficacy of Kwell Lotion as a scabicide and pediculicide makes possible, in most patients, complete eradication of either scabies or pediculosis by means of a single application. Kwell Lotion is non-irritant and does not lead to secondary dermatitis or skin irritation, even when applied to the skin of children or in the presence of secondary infection.

Kwell Lotion contains one per cent gamma benzene hexachloride incorporated in a water-miscible, greaseless and stainless lotion vehicle. Pleasantly scented, it offers the utmost in convenience and patient acceptability.

Available in 2 ounce and 1 pint bottles on prescription at all pharmacies.

CSC

KWELL OINTMENT

Kwell Ointment offers the therapeutic virtues of Kwell Lotion in a pleasantly scented vanishing cream base. It is useful especially in the treatment of scabies in younger children where prolonged skin contact must be maintained. Supplied in 2 ounce and 1 lb. jars.

C.S.C. Pharmaceuticals

A DIVISION OF COMMERCIAL SOLVENTS CORPORATION, 17 EAST 42ND STREET, NEW YORK 17, N.Y.

(In writing to advertisers, please mention the journal—it helps.)

Granular DIELDRIN-BENTONITE proved effective mosquito larvacide

● Field tests in Arkansas* rice fields with 5% Dieldrin in granular Bentonite at dosages as low as 0.05 pound to the acre gave 99 plus per cent control of mosquito larvae (*Psorophora confinnis*). Of great significance to mosquito control workers is the fact that only *one pound* of the 5% granular Dieldrin-Bentonite formulation to the acre was required to do the job. Following exposure to weathering in the field Dieldrin retained its toxicity better than any other insecticide tested.
Important advantages of granular Dieldrin-Bentonite for control of mosquitoes and other water-borne pests such as Culicoides (salt marsh sand flies) are:

(1) Dieldrin-Bentonite granules penetrate the vegetative canopy often found above mosquito breeding areas.

(2) This new type formulation eliminates waste. A higher percentage of the toxicant in the bentonite granules reaches the infestation level regardless of cover density than other in types of formulations.

(3) The granules of this formulation start to deflocculate immediately upon contact with water, releasing the toxicant when and where it is needed for mosquito larvae control.

(4) Granular Dieldrin-Bentonite does not drift, thus enabling airplane application from greater altitudes, with more accuracy.

(5) Granular Dieldrin-Bentonite can be applied under conditions of greater air movement than dust and/or sprays. Hence, it can be applied many more hours a day with attendant economies and other advantages.

(6) Dieldrin mixed with Bentonite granules reduces toxic hazard of handling to a minimum. Skin contact and inhalation risk are almost wholly eliminated.

(7) Granular Dieldrin-Bentonite stores well.

This new type formulation is now available for experimental work; we invite you to investigate this economical modern method of applying Dieldrin where heavy vegetation presents a problem. Write today for complete information.

Julius HYMAN & Company
DENVER, COLORADO

*Bulletin 511, dated June, 1951, "Rice Field Mosquito Control by Pellet-Borne Insecticides," by F. E. Whitehead, Agricultural Experiment Station, University of Arkansas College of Agriculture, Fayetteville, Arkansas.

(In writing to advertisers, please mention the journal—it helps.)

Foreman instructing native spray-hands in the cleaning of their equipment, during PHAS Iran Project.

A SERVICE DESIGNED TO HELP YOU

Pennsalt Health and Agriculture Service

To implement a successful malaria control program, the malariologist has a skilled nucleus of men who work as a team—in training local workers, analyzing disease vectors, organizing and directing the actual control work. However, should another outbreak demand a new program or intensified control efforts, you may need the services of a new and complete "team." The Pennsalt Health and Agriculture Service—known as PHAS—was organized to provide you with just such a nucleus of trained technicians and administrators ready for assignment anywhere in the world.

In order to help you complete programs as rapidly as possible, a panel of experts is available to PHAS, in addition to the regular staff, for consultation or field aid on specific projects. These specialists include entomologists, public health and sanitation engineers, agricultural experts and many others.

A striking example of the kind of service PHAS offers is provided by the recent malaria-control project assigned to PHAS, in the Shiraz Valley of Iran. Sponsored by the Iran Foundation in cooperation with Iranian government authorities, the project's aim was to control malaria-bearing mosquitoes in a selected area. Beginning on May 27th, 1950, the PHAS team trained 42 supervisors and 100 spray hands, analyzed the malaria vectors, and directed the spraying of 350 villages—nearly 6 times the number contracted for. In addition the project was completed by September 1st—far ahead of schedule. (Although lack of data on prior malaria incidence prevented a scientific evaluation, public reaction to the program was enthusiastic.) Many village head-men reported no new cases of malaria and a marked drop in mosquito infestation after the spraying.

This Iran project has been fully written up in a 35-page illustrated report. If you would like a free copy, just write to PHAS, in care of the address below. And, if you have a specific pest control problem, why not talk it over with us to find out how PHAS can help you. You will be under absolutely no obligation to buy any Pennsalt chemicals . . . all PHAS offers is technical know-how. Let us hear from you.

PENNSALT HEALTH AND AGRICULTURE SERVICE

PENN SALT

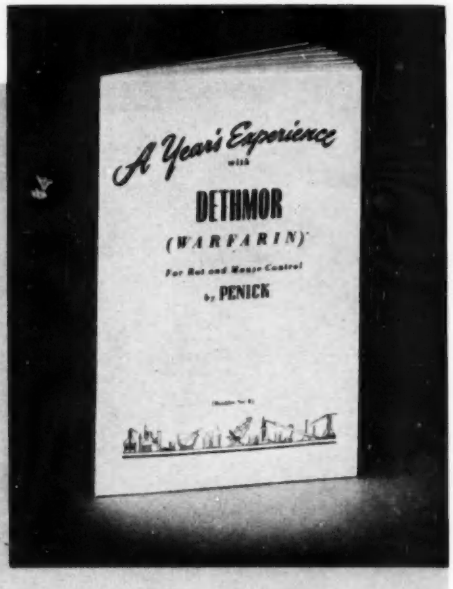
Widener Building, Philadelphia 7, Pa., U.S.A.

(In writing to advertisers, please mention the journal—it helps.)

Write for this 36 Page Booklet about DETHMOR--

The incidence of plague and typhus fever are fairly well tabulated — but behind the tables are the millions of rats which can quickly transform endemic plague and typhus to pandemic.

Consider the unrecorded millions of cases of salmonellosis, trichinosis, hemorrhagic jaundice and rat-bite fever for which the rat is responsible.



Dethmor (warfarin) provides public health workers with a new effective weapon against rats.

Warfarin has proved to be a remarkable rodenticide — entirely different from previous materials. Millions of pounds of bait have been used for the control of rats and mice under every possible circumstance. Results are consistently outstanding.

The reports which we have received from users have been impressive. We felt it important to summarize their experience and present it to those interested in warfarin. The result is "A Year's Experience with Dethmor (Warfarin)."

Particularly pertinent are the remarks of the U. S. Public Health Service regarding their experimental work for the control of Norway rats with warfarin in a focus of murine typhus.

TO CONTROL RAT BORNE DISEASES CONTROL RATS
WITH DETHMOR (WARFARIN)

S. B. PENICK & COMPANY

50 CHURCH STREET, NEW YORK 7, N. Y.
Telephone, COrtlandt 7-1970

735 WEST DIVISION STREET, CHICAGO 10, ILL.
Telephone, MOHawk 5651

Use

DDT

DICHLORODIPHENYLTRICHLOROETHANE

EASY
APPLICATION

EFFECTIVE
CONTROL

AVAILABLE
FOR PUBLIC
HEALTH USE

- Technical Grade DDT
- 25% Easy Emulsifiable Oil Concentrate
- 75% DDT Wettable Powder (high suspension)
- 50% DDT Wettable Powder
- 75% DDT Dust Concentrate
- 50% DDT Dust Concentrate
- Technical Grade BHC

Write or Wire

MICHIGAN CHEMICAL CORPORATION

TRADE MARK

MICHIGAN CHEMICAL CORPORATION
ST. LOUIS, MICHIGAN Cable Address: MICHEMCO

USE HUDSON TESTED and PROVED SPRAYERS

SAVE TIME . . . SAVE WORK!

HUDSON "SIMPLEX" INDUSTRO SPRAYER

"The Higher the Pressure—The Tighter the Seal!" Cover slips INSIDE large 3¾ x 4¼" opening of tank for more secure sealing. Pump is separate unit assuring longer wear. Available in galvanized or stainless steel. Rotatable spray control valve, adjustable nozzle. Equipped with 5 feet of hose and pressure gauge. 4 and 2¾ gallon capacity.

HUDSON SPRAZIT KNAPSACK SPRAYER

Easily maintains 80 pounds continuous nozzle pressure. 5½ gallon capacity galvanized or stainless steel. Rotatable spray control valve, 24" extension spray tube and 60 inch spray hose. Wide, adjustable, well-padded straps.

HUDSON PEERLESS POWER SPRAYERS

A complete line with tank capacities in 15, 30, 50, 100 and 150 gallons, wheel or skid mounted. Output of 4, 5 or 8 G.P.M. Pressures 0 to 400, 0 to 500 and 0 to 800 pounds. Write for further details.

HUDSON
Tested and Proved EQUIPMENT

H. D. HUDSON MANUFACTURING COMPANY

589 E. ILLINOIS ST., CHICAGO 11, ILLINOIS

For Fly
and Mosquito Control

VELSICOL 1068
CHLORDANE

Extensive laboratory and field tests, during the past six years, have established the effectiveness of chlordane in the control of flies, outside of residences.

A 2½% spray is effective.

In addition to showing outstanding control of adult flies, chlordane has given effective results as a fly and mosquito larvacide when applied to their respective breeding places.

Complete information as to proper applications and formulations gladly sent upon request.

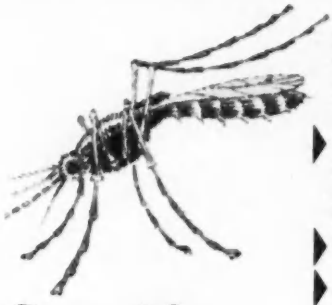
VELSICOL CORPORATION

General Offices and Laboratories
330 East Grand Avenue, Chicago 11, Illinois

Export Division
100 East 42nd Street, New York 17, New York

REPRESENTATIVES IN PRINCIPAL CITIES

FOR YOUR MOSQUITO CONTROL PROGAM



In every field of pest control, General Chemical insectides and fungicides have earned a reputation for consistently high performance. That's because each is backed by—

- Nearly fifty years' experience in the production of insecticidal materials . . . and by close association with pest control authorities in their use.
- Extensive research in laboratory and field.
- Continuous production control for highest quality and consistent uniformity.

Specify

GENERAL CHEMICAL

DDT: Technical Grades, Finely Ground and Flake • High Concentrate Wettable • High Concentrate Dust Base • Solvent Concentrates • Emulsifiable Concentrates • Special Formulations

BHC: Technical Grades • Finely Milled Dust Base Concentrates • Wettable Concentrates • Special Formulations

LINDANE: Technical Grade • Wettable Concentrate • Emulsifiable Concentrate

Other General Chemical pest control materials include: Special Formulations of Toxaphene, Aldrin, Dieldrin; Parathion; Arsenicals; other organic and inorganic insecticides and fungicides.

BASIC CHEMICALS

GC

FOR AGRICULTURE

©

For Weed Control:
2,4-D Esters and Amine
2,4,5-T Brush Killer

GENERAL CHEMICAL DIVISION
ALLIED CHEMICAL & DYE CORPORATION
40 Rector Street, New York 6, N. Y.
Offices in Agricultural Centers from Coast to Coast

TO ASSIST YOU IN SPECIFYING STAINS, INDICATORS AND LABORATORY REAGENTS

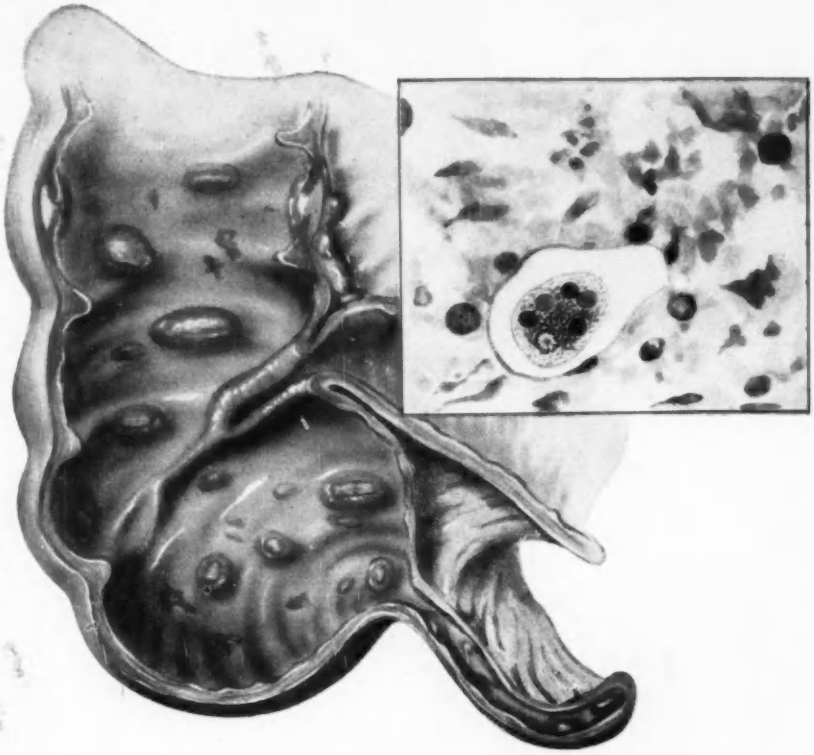
We will be pleased to send a copy of our current *60-page Catalog and Price List* which enumerates our complete line of Biological and Laboratory Reagents; Vital Stains; Biological Stains and Reagents; Commission Certified Biological Stains; Staining Solutions and Reagents in Solutions; National Indicators; Clark and Lubs Indicators; Oxidation-Reduction Indicators, and Water Soluble pH Indicators. This useful book also contains a useful Hydrogen Ion Indicator Chart as well as other useful information.

PHARMACEUTICAL LABORATORIES

NATIONAL ANILINE DIVISION

ALLIED CHEMICAL & DYE CORPORATION

40 RECTOR STREET, NEW YORK 6, N.Y.



in amebiasis:

"In hospitals and institutions where the incidence of amebiasis is significant, simultaneous mass treatment by terramycin can control the infection . . . stool examinations performed 6 months after terramycin therapy revealed an apparent effectiveness of 100 per cent."

Most, H.; Tobie, J. E.; Bozicevich, J., and Reardon, L. V.: cited in Pub. Health Rep. 65:1684 (Dec.) 1950.

CRYSTALLINE TERRAMYCIN HYDROCHLORIDE

available | *Capsules, Elixir, Oral Drops, Intravenous, Ophthalmic Ointment, Ophthalmic Solution.*

ANTIBIOTIC DIVISION Pfizer CHAS. PFIZER & CO., INC., *Brooklyn 6, N. Y.*

ANNOUNCING A NEW JOURNAL

This is the first issue of the American Journal of Tropical Medicine & Hygiene, which takes the place of the American Journal of Tropical Medicine and the Journal of the National Malaria Society. The fusion came about quite naturally as a consequence of the amalgamation of the two Societies of which these journals were the official organs. The reasons for such a step are convincingly set forth in Dr. Justin Andrews' Presidential Address to the National Malaria Society, which will be found on another page. Hundreds of us who were members of both Societies have welcomed this important event in the world of tropical medicine as a sign of the times. Both the perpetuation and the blending of interests and activities which this union brings about are now particularly desirable and opportune.

For the disappearance of malaria as a public health problem in the United States, and the dissolution of the National Malaria Society does not signify in any way a cessation of interest in it as a disease. Malaria still remains the most destructive enemy of human rural life and economy in three quarters of the world. In field and laboratory we are pressing to find answers to new questions and old problems which challenge the epidemiologist, laboratory investigator, entomologist, and engineer in any part of the globe, since these, whether in war or peace, have now become matters of concern to us all.

But more important than the geographical oneness of the malaria problem has been its progressive integration with all the other problems and activities which have a bearing on the health of mankind in the tropics. This interdependence has been recognized for some time and was illustrated by the fusion of the two Congresses on Tropical Medicine and Malaria, which were separate entities at the meetings in Washington in 1948 but which will convene as one hereafter. The two Societies have now embodied this idea in a joint corporation, and they have also desired to emphasize, by a change in name, the public health objective which has in fact always been the implicit mainspring of their existence.

Thus the new Journal more comprehensive than the old in the expanded compass of its interests, hopes to offer its readers a broader sweep and richer content than before, and to provide a channel of communication to the workers in every field of tropical medicine and hygiene.

THE EDITOR

FOREWORD

Upon the death of Charles Franklin Craig on December 9, 1950, a group of his colleagues and former students, members of the American Society of Tropical Medicine, proposed that a special number of the American Journal of Tropical Medicine be dedicated to his memory in recognition of his great services to the Journal and to the Society, and this was approved by the Council.

Dr. Craig had been Colonel of the Medical Corps, United States Army; Commandant of the Army Medical School; and Professor of Tropical Medicine at George Washington University and at Tulane University Medical Schools. Thus he was bound by the closest ties to both the military and civilian medical circles of our country, and in both, for his unusual abilities as teacher, author and investigator, he came to occupy a position of eminent authority.

But overlapping these careers and extending well into his retirement, his editorship of the American Journal of Tropical Medicine for two decades, from 1927 to 1946, developed into an outstanding accomplishment. It brought success and prestige to the Journal during its most difficult years; it provided a stimulant and an outlet to early workers in tropical medicine which was a field little cultivated at that time by our medical profession; and it exerted a sound influence on the growth and development of our Society.

With the merger of the American Society of Tropical Medicine and the National Malaria Society, it seems fitting that the first number of the American Journal of Tropical Medicine and Hygiene should be the one to commemorate Colonel Craig whose creative work is built into the very foundations of our knowledge of malaria and of so many other fields of tropical medicine.

The papers gathered in this issue touch on a wide diversity of problems in which Colonel Craig took an active interest to the very end of his life, and the authors include distinguished representatives not only of the Army and the civilian medical schools to which the Colonel devoted his active years, but of many other medical and public health institutions and organizations as well, which have desired to honor him.

The Committee, in the name of the Society, wishes to express its appreciation to the authors for their contributions to this memorial number of the Journal, dedicated to one who for more than fifty years devoted his exceptional intelligence, experience and judgment to advancing our knowledge, indoctrinating our youth and disseminating the results of their investigative activities in the field of tropical medicine.

W. A. Sodeman
George R. Callender
James S. Simmons
L. W. Hackett

Charles Franklin Craig
1872–1950

CHARLES FRANKLIN CRAIG: FIFTY YEARS OF WORK AND SERVICE IN TROPICAL MEDICINE

C. C. BASS

Tulane University, School of Medicine, New Orleans, Louisiana

When your committee invited me to prepare and deliver this, the sixteenth Charles Franklin Craig Lecture, my first reaction was to decline the honor, knowing full well that I could not hope to present an address to equal some of the previous masterful lectures of this series. However, since it would afford an opportunity to remind ourselves of the vast amount of valuable work and service in the field of tropical medicine done during the past half century by a worthy contemporary and friend, there was no choice but to accept.

The medical career of Charles Franklin Craig began towards the close of the nineteenth century. He was born and reared at Danbury, Connecticut, and received his medical degree from Yale University in 1894. Following his graduation, he returned to Danbury to practice. The Spanish-American war came in 1898 and was followed by several years of occupation and the rehabilitation of the health and welfare of the people of tropical lands which had been liberated from long years of tyranical Spanish domination and misrule. There was the problem also of the diseases which are more prevalent in warm countries, acquired by soldiers and others during their sojourn abroad and brought back to this country, as an added health menace and a challenge to the health service and the research ability of American medical scientists. Tropical diseases in general became much more important and of much more interest to American Medicine.

Any of us can recall many incidents and happenings which turned our interests and our lives in one direction or another. Some of them have been more or less personal or local. Often they seemed quite unimportant at the time. Had they not happened, however, our lives would have been different, either for better or for worse. Some of such occurrences took place far beyond our own immediate environment. The coming of the Spanish-American war in 1898, which was touched off by the sinking of the U. S. battleship Maine in Havana harbor on 15th of February, 1898, provided the opportunity which led our young hero into his long useful career in tropical medicine. Had that war not come, things would have been different in many respects.

Young Craig became a contract surgeon with the United States Army in 1898, serving first at Sternberg General Hospital (Chicamauga Park, Georgia), then during the next three years successively at Simpson General Hospital (Fortress Monroe, Virginia), Camp Columbia (Havana, Cuba) and the Army General Hospital (Presidio, California). In 1903, he became a regular Army officer, starting as first lieutenant, and through succeeding promotions attained the rank of Colonel in 1918, which he held until his retirement from the Army in 1931. During his long term in the regular Army, he served in posts in Manila with the Army Board for the study of Tropical Diseases; in Hawaii; at El Paso,

Texas; at Ft. Leavenworth, Kansas; and in Washington, D. C., at different times, in the Army Medical Museum, the Army Medical School and the Army Medical Center. After his retirement from the Army in 1931, he became Professor of Tropical Medicine and head of the department at Tulane University, which position he held until his retirement in 1937. Following his retirement from Tulane University he moved to San Antonio, Texas, where he lived and continued his work and writing in the field of tropical medicine until he died on the 9th of December, 1950.

The period covering the first decade of Craig's medical career was a most momentous one for medicine in general, and for tropical medicine especially. Laboratory methods of medical research and diagnosis were developing and use of the microscope in the diagnosis and study of tropical diseases was rapidly replacing previous inaccurate methods. This was especially important regarding malaria. Only through microscopic examination of the blood and identification of the parasite could the former guesswork about the disease be replaced by accurate information.

By the same means, the specific agents or parasites of several other diseases occurring principally in tropical countries have been discovered and accurate diagnosis and study of them has taken the place of previous inaccurate and wrong beliefs. Fortunate indeed was any ambitious worker who at that time realized the need for, and learned to use the microscope and laboratory methods in his work. This was particularly true for anyone who was interested in malaria, the principal disease to which Craig devoted a large part of his studies and attention during the early years of his tropical medical career. It was an exciting period in medical history.

Throughout the history of medicine, individuals have set up milestones of fundamental discoveries for those who come after them. When such milestones are set upon firm foundation they serve as guide posts by which others must pass, as they advance to still other information. Such first step mileposts in the field in which we are now thinking include the discovery of the malaria parasite by Charles Louis Alphonse Laveran on November 6, 1880; Manson's discovery of the passage of filaria through the stomach wall of the mosquito into the abdominal cavity, and thence into the thoracic muscles, during which time it increased in size, developed a mouth, an alimentary canal and other organs; discovery of transmission of Texas cattle fever by the cattle tick, by Theobald Smith in 1891; observation by Ronald Ross, on August 20, 1897 of a malaria parasite growing within the stomach wall of a mosquito which had fed exclusively on a patient whose blood contained crescents; observation of ex-flagellation of a malaria-like organism in blood from a crow, by W. G. McCallum in 1897, and observation of the penetration and thus fertilization of another malaria parasite in the blood of man, by McCallum and Opie in 1898; demonstration of the entire life cycle of the parasite of bird malaria, from bird to mosquito and in turn from moquito to bird, by Ronald Ross on July 9, 1898; and demonstration of the entire life cycle of the parasite of human malaria from man to mosquito and from mosquito to man, in November, 1898, by Amico Bignami. Note that it took

almost 20 years, from the discovery of the malaria parasite in man in 1880, to establishment of the mode of transmission by mosquitoes from man to man, in 1898.

The circumstances and needs in the early years of the 20th Century and just following the Spanish-American war, provided the opportunity for practical application of the fundamental information that had only recently been established relative to tropical diseases. One of the outstanding historical events was the sanitation of the city of Havana, Cuba, under the direction of that great benefactor to mankind, William Crawford Gorgas. Within a short period of well organized and well executed sanitation, this veritable pest-hole of disease and unsanitary conditions was converted into the cleanest and most healthful city in the Western Hemisphere.

At about this same time favorable circumstances were provided in Cuba for the necessary experimental work, demonstration and proof, for all time, of the transmission of yellow fever by mosquitoes, by Walter Reed and his co-workers.

The French attempted to build the Panama Canal and failed. They left fifty thousand tombstones and mountains of abandoned machinery and equipment as the most convincing evidence of the supremacy of the diseases of the tropics, especially malaria and yellow fever, over man at that time. Throughout history one or the other of these diseases had prevented the advancement of civilization in many parts of the world or had caused the decline, sooner or later, of civilizations that had arisen at a more favorable time.

Building of the canal later in this same early part of the 20th Century, by the United States, was made possible by the control of these diseases under the direction of the immortal Gorgas. This greatest of all demonstrations, up to that time, of large scale control of disease and maintenance of health in the heart of the tropics, was accomplished by the work of a corps of able assistants and lieutenants on Gorgas' staff, too numerous to name here, most of whom are no longer with us. I should like to mention one here, that indomitable worker and expert sanitary engineer Mr. J. A. LePrince, whose work and perseverance contributed largely to the spectacular results of sanitation whereby not only malaria but many other diseases were rapidly reduced and kept under control in vital areas during the building of the canal. It is a pleasure to note that on yesterday the National Malaria Society presented the Joseph Augustin LePrince award to this distinguished worker, in well deserved recognition of his useful service.

Before we take up the work and service of Charles Franklin Craig in tropical medicine, which may be said to have begun in 1898, the year he volunteered as a contract surgeon in the U. S. Army, it may be interesting to go a little further back and see what he was doing that led him towards the field of his long useful career. During the four years following his graduation, he published a total of 12 papers on a variety of medical subjects. Most of them were of interest in general medicine and nearly all of them dealt principally with the laboratory or bacteriological side of the subject. The very first one was a report of a case of umbilical hemorrhage which arose in his practice. Other subjects were bac-

teriology of tuberculosis; parasitic origin of carcinoma; morphology of tubercle bacillus; the uric acid diathesis; pathology and bacteriology of appendicitis; bacteriology of diphtheria; bacteriology of epidemic parotitis, with description of a diplobacillus found in the blood and urine; Widal's test for typhoid. But the paper of most importance, in point of experience and qualification for advancement, when the opportunity came, was published in 1896, entitled "Recent Advances in Our Knowledge Concerning the Malarial Organism".

This paper shows that the author had a wide knowledge of what was known on the subject at that time. He described methods of diagnosing the parasite, structure of the organism, the many forms found, the relation of the organism to the various types of malarial fever, and the crescentic and flagellate bodies. The author's personal knowledge of the parasite and the kind of work he did are indicated by some 15 figures consisting of splendid original drawings of different stages and forms of the organism. His wide acquaintance with the literature and his reliance upon it for information are indicated by the total of 52 references cited.

Another paper, the preparation of which no doubt helped to lead him in the direction of his life's career, was published in 1898 but probably prepared earlier, entitled "Transmission of Disease by the Mosquito". There was another one later which was intended as a continuation or complement to this one, entitled "Transmission of Disease by Certain Insects: Ticks, Bedbugs, Ants, etc."

The research and work in preparing the three papers just mentioned brought the writer in touch with the discoveries, the methods and the theories of the great of that day—Laveran, Manson, Ross, Theobald Smith. Other contributors in the field were Thayer, Dock, McCallum, Bignami, Marchiafava, Celli, Grassi, Ziemann, Koch and many others.

Craig was profoundly impressed when he read, for the first time, the classical work of Theobald Smith and the monograph by Smith and Kilborn upon the nature, causation and prevention of Southern cattle fever. It is impossible for one to read this monograph without admiring and profiting by the scientific, accurate methods by which the authors approached their subject and finally proved their thesis. It will always remain a model of scientific research.

This was the first instance, in all history, proving the transmission of a specific disease-producing parasite from animal to animal through the bite of an arthropod. Although its great importance has been largely forgotten because of later and more dramatic discoveries, such as the transmission of malaria by mosquitoes, it is undoubtedly true that the stimulus furnished by these researches to the study of the transmission of disease, was very largely responsible for the later discoveries regarding the relationship of mosquitoes to malaria, yellow fever and dengue; of flies to trypanosomiasis; of fleas to plague; of ticks to Rocky Mountain spotted fever; of lice and fleas to typhus fever; and of a long, and still increasing, list of other insects and diseases.

Imagine our young medical scientist, eager for knowledge, ideas and methods of research that might lead to clarification of the mode of transmission of the great crippler and killer, malaria, being thrilled and stimulated by his acquaint-

ance, through this contribution on Southern cattle fever especially, with one of the greatest scientists of his time. It was particularly fitting that the Theobald Smith medal of the American Academy of Tropical Medicine was awarded to Col. Craig in 1943. This medal was provided to honor the name of the great man whose research and example helped so much to kindle the fire of ambition and hope in the mind of our young researcher and which doubtless contributed, in no small way, to lead him quite naturally into the field of tropical medicine, to which he contributed so much during his long career.

Craig was Director of the Bacteriological Laboratory at Sternberg General Hospital in 1898 and was associated there with George Dock, one of the authorities, at that time, on malaria. Dock was an able clinician, with laboratory background. Their joint interests in both malaria and typhoid brought them closely together. In accordance with my own experience, any young man who worked around and with men like Thayer and McCallum and Dock, would surely catch the fire that such men kindle in their associates.

Here large numbers of soldiers were suffering with fevers which, as was commonly the case at that time, were usually misdiagnosed and mistreated as malaria or typho-malaria, the latter a term then still in general use but soon to be abandoned. Young Craig knew his malaria parasites and how to find them in blood from patients. He also knew how to make the Widal test for typhoid, having previously published papers on each of these subjects. Therefore he was prepared to apply these accurate laboratory diagnostic methods, in sharp contrast to other inaccurate, guesswork methods. In this instance, much of the fever proved to be typhoid, spread by contamination of food and water due to unsanitary conditions in and around the camp.

During and following the Spanish-American war, Craig had extensive experience in studying malaria parasites in the blood, and the clinical symptoms of the different forms of malaria in hundreds of soldiers at the several Army hospitals where he served. The abundance of material provided ideal conditions for the diligent student of malaria to observe what goes on. During the early years of the present century, he became well known as an authority on malaria. He published many papers on the subject and two most useful authoritative books in 1901 and in 1909.

Towards the second decade of the present century, Craig became more and more interested in amebiasis, a subject to which he devoted much study and research, and contributed much information. He published three authoritative reference books on this subject in 1911, 1934 and 1944, in addition to numerous papers. He, more than anyone else kept the dangers of amebiasis before the American physicians. Attention was directed to the wide prevalence of this infection, frequently without recognizable symptoms that could be identified with it.

He was a strong advocate of good preparation of material, fixation and staining, by which the parasite could be recognized and identified. Differentiation of the prevalent pathogenic *histolytica* species from other non-pathogenic or rarer forms also requires refined technic such as he advocated.

He perfected the complement-fixation test for amebiasis. This was a logical

interest, since he had previously become a skilled serologist and had published a laboratory manual on the Wassermann test in 1918. Whenever suitable cultures became available as a source of the necessary antigen, it was possible for him to carry out the necessary experimental work to develop the test and determine its usefulness. As an authority on amebiasis he was one of several specialists selected to investigate the epidemiology of an unusual outbreak of the disease that occurred, in 1934, in the hotel (The Congress) in which we are meeting today.

Craig was an inspiring teacher and an able writer. In order to record a correct picture of his contributions to the literature, I have gotten together and append hereto, his complete bibliography during his entire medical career. There are a total of 226 titles which include 7 books and 11 sections in medical textbooks. Fifty-two of the titles indicate an application to malaria and 78 to amebas or amebiasis. In addition to these two disease categories in which he was best known, a large part of his other publications apply, either directly or indirectly, to a considerable number of other diseases which fall in the general field of tropical medicine.

Now let us direct attention to another excellent service of Craig to the field of tropical medicine, for which the American Society of Tropical Medicine should be forever grateful. For almost two decades, 1927–1946, he served as editor of the American Journal of Tropical Medicine. He was well qualified for this service because of his broad experience and previous long continued, almost undivided, interest in the field, to the literature of which he had been a large contributor. He knew what had been done in tropical medicine and who had contributed the information. Through his acquaintance with the literature and personal acquaintance with the workers, he knew much of the research, promotional and teaching activities in the field. With his background of experience and his aptitude for writing, he was well qualified to evaluate material submitted for publication, and especially to do the thankless, never ending editorial work required.

During the period of his editorship, the journal progressed from a struggling, small-sized publication with a small subscription list, to a yearly total of 800 to 900 pages and a substantially increased number of subscribers. A volume has been published every year. Thanks largely to the work of Craig as editor and often as contributor, the entire collection of 31 completed volumes comprises a valuable storehouse of information on American tropical medicine, recording much of the work and progress in the field to this good day. It will be a challenge to succeeding editors to maintain, in the future, the high standard set during Craig's editorship.

Doctor Craig was small in stature and frail in appearance. One wonders how he was able to accomplish so much, and to contribute so greatly in his field, as compared with many others endowed with much greater physical strength and vigor. One thing that may have contributed to the accomplishment of so much useful writing was the method by which he carried out such work. If we consider all that he ever wrote, including scientific papers, books and sections in textbooks edited by others, I estimate that it would add up to more than 10,000 pages, at

least 90% of which can be definitely placed in the field of tropical medicine. This meant much time and labor spent in the preparation of such an amount of manuscript.

During his 7 years at Tulane, I often had occasion to see him going on with his work quietly, as if there were no haste or worry whatever. A favorite habit, when writing or revising previous writings, was to assign himself, for a given day or period, a task of a certain number of pages, or the completion of a certain part of the subject. After the task was done, he was ready to quit and relax until time to take up another. He never seemed to be under any great strain or worry to meet a deadline which was crowding him. He seemed to plan his work and time too well for that.

In conclusion, we are reminded of fifty years of useful and successful work and service, in the field of tropical medicine, by a brilliant student, an inspiring teacher and an able writer—Charles Franklin Craig.

BIBLIOGRAPHY OF CHARLES FRANKLIN CRAIG, M.D.

I. Books.

1. The Estivo-Autumnal (Remittent) Malarial Fevers. New York, W. Wood & Co., 1901.
2. The Malarial Fevers, Haemoglobinuric Fever and the Blood Protozoa of Man. New York, W. Wood & Co., 1909.
3. The Parasitic Amoebae of Man. Published with the authority of the Surgeon General of the United States Army, Phila. & London, J. B. Lippincott, 1911. 253 p.
4. Studies of Syphilis, with introduction by Major Frederick F. Russell, by Charles F. Craig and Henry J. Nichols. Published for the information of Medical Officers, with the approval of the Secretary of War, Washington Government Printing Office, 1913.
5. The Wassermann Test. St. Louis, C. V. Mosby Co., 1918, 2nd ed., 1921.
6. A Manual of the Parasitic Protozoa of Man. Phila. & London, J. B. Lippincott Co., 1926. 569 p.
7. Laboratory Methods of the United States Army. Phila., Lea & Febiger, 1929.
8. Amebiasis and Amebic Dysentery. Springfield, Ill., Charles C. Thomas, 1934. 315 p.
9. Clinical Parasitology, by Charles Franklin Craig and Ernest Carroll Faust. Phila., Lea & Febiger, 1st ed., 1937; 2nd ed., 1940; 3rd ed., 1943; 4th ed., 1945; 5th ed., 1951.
10. Laboratory Diagnosis of Protozoan Disease. Phila., Lea & Febiger, 1942. 2nd edition. Phila., Lea & Febiger, 1948, 384 p.
11. The Etiology, Diagnosis and Treatment of Amebiasis. Baltimore, Williams & Wilkins, 1944.

II. Scientific Papers

12. A study of the microscopic phenomena of inflammation with special reference to diapedesis of the white blood corpuscles, *Proc. Am. Micr. Soc.* **16:** 165–179, 1894.
13. Umbilical hemorrhage; etiology, pathology and treatment; report of a case, *Med. News*, Phil. 569–570, Nov. 24, 1894.
14. Bacteriology of tuberculosis; historical, practical and experimental, *New England M. Month.* **15:** 301–304, 1896.
15. Action and uses of kola, *New England M. Month.* **15:** 356–358, 1896.
16. Parasitic origin of carcinoma, *Proc. Connect. M. Soc.* p. 204–211, 1896.
17. Recent advances in our knowledge concerning the malarial organism, *Med. Rec.* (N. Y.) **50:** 664–670, Nov. 7, 1896.

18. On Widal's method of diagnosticating typhoid by means of blood serum, *New York M. J.* **65:** 186–188, Feb. 6, 1897.
19. On variations in the morphology of the bacillus tuberculosis, *Medicine* **3:** 462–467, June, 1897.
20. Some remarks upon the uric acid diathesis, and its treatment, *New England M. Month*, **16:** 271–275, 1897.
21. The bacteriology of tuberculosis, *Proc. Connect. M. Soc.* p. 203–212, 1897.
22. Notes on pathology and bacteriology of appendicitis, *New England M. Month.* **16:** 407–417, 1897.
23. Diphtheria: its bacteriology, *Tr. Am. Micro. Soc.* (1896) **18:** 271–281, 1897.
24. Bacteriology of epidemic parotitis, with description of a diplobacillus found in blood and urine, *Yale M. J.* **4:** 319–323, 1897–98.
25. The branched form of the bacillus tuberculosis in sputum, *J. Exper. Med.* **3:** 363–370, 1898.
26. The transmission of disease by the mosquito, *New York M. J.* **67:** 377–381, March **19:** 453–457, April 2, 1898.
27. The transmission of disease by certain insects; ticks, bedbugs, ants, etc., *New York M. J.* **68:** 593–599, Oct. 22, 1898.
28. Case of typhoid fever associated with quartan malaria, *Phil. M. J.* **3:** 640, March 25, 1899. (Abstract of paper read before Johns Hopkins Hospital Medical Society.)
29. Report of a case of combined typhoid fever and quartan malarial fevers, *Phil. M. J.* **3:** 1369–1371, June 17, 1899.
30. Observations upon flagellated malaria plasmodia; description of two varieties, *New York M. J.* **70:** 913–917, Dec. 23, 1899.
31. The parasites of estivo-autumnal (remittent) malarial fever, *Phil. M. J.* **5:** 764–768, April 7, 1900.
32. Estivo-autumnal malaria, *Med. News* **76:** 934, June 9, 1900. (Abstract)
33. Bubonic plague from a sanitary standpoint, *Pacific M. J.* **43:** 577–597, August, 1900.
34. The flagellated malaria organism (Abstract), *Brit. M. J.* **2:** 1037, Oct. 6, 1900.
35. Observations on a spirillum occurring in false membrane of the tonsil, *Med. News* **76:** 374–375, Nov. 3, 1900.
36. Observations upon the quartan malarial parasite and upon the staining reactions of the tertian, quartan and estivo-autumnal parasites, *Med. News* **77:** 681–685, Nov. 3, 1900.
37. Report on the work done in the Bacteriological Laboratory of the United States Army General Hospital, San Francisco, Cal., *Rep. Surg. Gen. U. S. Army*, pp. 53–71, 1900.
38. Estivo-autumnal malarial fever, *J.A.M.A.* **35:** 1139–1148, Nov. 3, 1900.
39. Observations upon the amoebae coli and their staining reactions, *Med. News* **78:** 414–418, March 16, 1901.
40. The parasites of malarial fever (reviewed), *Med. Rec.* **61:** 700–701, May 3, 1902.
41. A new method of staining the malarial parasites with a description of the staining reactions, *New York M. J.* **76:** 451–453, Sept. 13, 1902.
42. A case of chronic lymphatic leukaemia with lymphosarcoma or myeloma of the sternum and ribs, *Med. News*, Oct. 4, 1902.
43. Pathology of latent malarial infection as observed at autopsy, *J. Ass. Mil. Surg.* **13:** 381–388, 1903.
44. Latent and masked malaria (Abstract), *Rep. Surg.-Gen. U. S. Army*, pp. 67–68, 1902.
45. Malta fever; its occurrence in the United States Army with a review of the literature, *Am. J. Med. Sci.* **125:** 105–115, Jan., 1903.
46. Pathology of latent malarial infection as observed at autopsy. *Am. Med.* **6:** 145–147, July, 1903.
47. Malarial infections; their parasitology, symptomatology, diagnosis and treatment, *Internat. Clin.* 13 ser. v. **3:** 159–192, 1903.

48. The occurrence of uncinariasis (ankylostomiasis) in soldiers of the U. S. Army, *Am. J. Med. Sci.* **126:** 798–816, Nov., 1903.
49. Observations on Malta fever in the U. S. Army, *J. Ass. Mil. Surg.* **15:** 264–281, 1904.
50. The life cycle of *Amoeba coli* in the human body; a preliminary report, *Am. Med.* **7:** 299–301, Feb. 20, 1904.
51. Rupture of the spleen, *Med. News* **84:** 780–783, April 23, 1904.
52. The pathology of chronic specific dysentery of tropical origin, *J. Ass. Mil. Surg. U. S.* **14:** 350–378, June, 1904.
53. The complications of amoebic and specific dysentery as observed at autopsy; an analysis of 120 cases, *Am. J. Med. Sci.* **128:** 145–156, July, 1904.
54. Classification of *Amoeba coli*, *Am. Med.* **8:** 185–186, July 30, 1904.
55. Observations upon latent and masked malarial infections with an analysis of 395 cases, *Am. Med.* **8:** 757–761, Oct. 29, 1904.
56. The relation of the so-called *Piroplasma hominis* and certain degenerative changes in the erythrocytes, *Am. Med.* **8:** 1016–1017, Dec. 10, 1904.
57. The etiology and pathology of amoebic infection of the intestine and liver, *Internat. Clin.* ser. 14, v. **4:** 242–298, 1905.
58. Observations upon the morphology and classification of the *Mycobacterium* (*Bacillus*) *tuberculosis*, *Med. News* **86:** 340–343, Feb., 1905.
59. Etiology and the pathology of bronchopneumonia complicating measles, *J.A.M.A.* **45:** 1187–1193, April 15, 1905.
60. Observations upon amebas infecting the human intestine with a description of two species, *Entamoeba coli* and *Entamoeba dysenteriae*, *Ann. Rep. Path. Lab. U. S. Army Gen. Hosp.*, San Francisco, 1905.
61. Idem. *Am. Med.* **9:** 854–861, May 8: 897–903, June 3; 936–942, June 10, 1905.
62. Intracorpuscular conjugation in the malarial plasmodia and its significance. *Am. Med.* **10:** 982–986, Dec. 9; 1029–1032, Dec. 16, 1905.
63. The action of quinine upon the tertian, quartan and estivo-autumnal malarial plasmodia, *Am. Med.* **1:** 7–13, April 13: 59–64, May 1, 1906. (Abstract in *Hyg. Zentralbl.* **2:** 505).
64. A new intestinal parasite of man: *Paramoeba hominis*, *Am. J. Med. Sc.* **132:** 214–220, Aug., 1906.
65. A new blood filaria of man: *Filaria philippinensis*. *Am. J. Med. Sc.* **132:** 435–443, Sept., 1906.
66. The symptomatology and diagnosis of Malta fever with the report of additional cases, *Internat. Clin.* 15 ser. v. **4:** 89–115, 1906.
67. Observations upon malaria; latent infection in natives of the Philippine Islands—intracorpuscular conjugations, *Philippine J. Sc.* **1:** 523–531, 1906.
68. A study of latent malarial infections, *Yale M. J.* **13:** 349, 1906–07.
69. Observations upon *Filaria philippinensis* in its development in the mosquito, *Philippine J. Sc.* **2:** 1-14, Jan., 1907. (Abs. Centralbl. Allg. Path. u. Path. Anat. **19:** 603–604, 1908) (With P. M. Ashburn)
70. A study of latent and recurrent malarial infection and the significance of intracorpuscular conjugation of the malarial plasmodia, *J. Infect. Dis.* **4:** 108–140, Jan., 1907.
71. Diagnosis of cholera, *J.A.M.A.* **48:** 692, Feb. 23, 1907. (With P. M. Ashburn)
72. Relation of intracorpuscular conjugation in the malarial plasmodia to latent and recurrent infections, *Internat. Clin.* 17 ser. v. **3:** 273–292, 1907.
73. Work of the Army Board for the Study of Tropical Diseases in the Philippine Islands, *Mil Surg.* **21:** 38–49, July 1907. (With P. M. Ashburn)
74. Tropical diseases in the Philippine Islands, *Mil Surg.* **21:** 140–148, Aug., 1907. (With P. M. Ashburn)
75. Tropical diseases as they exist in the Philippine Islands, *Mil Surg.* **21:** 220–225, Sept., 1907. (With P. M. Ashburn)

76. Tropical diseases in the Philippine Islands, *Mil Surg.* **21:** 336–348, Oct., 1907. (With P. M. Ashburn)
77. Tropical diseases as they exist in the Philippine Islands, *Mil Surg.* **21:** 527–530, Dec., 1907. (With P. M. Ashburn)
78. Study of tropical diseases as they occur in the Philippine Islands, Extracts from third quarterly report of the U. S. Army Board, *N. York M. J.* **85:** 1117–1119, 1907. (With P. M. Ashburn)
79. Experimental investigations regarding the etiology of dengue fever, with a general consideration of the disease, *Philippine J. Sc.* **2:** 93–152, 1907. (Also Abstract in *J. Infect. Dis.* **4:** 440–475, 1907. (With P. M. Ashburn)
80. The malarial fevers. In Osler's Modern Medicine, Editions 1, 2 and 3 (1907, 1914, 1926) Lea & Febiger, Phila.
81. Observations made during a visit to Japan by the U. S. Army board for the study of tropical diseases as they exist in the Philippine Islands, *Mil Surg.* **22:** 116–136, Jan., 1908. (With P. M. Ashburn)
82. A comparative study of Tsutsugamuschi diseases and spotted tick fever of Montana, *Philippine J. Sc.* **3:** 1–29, Jan., 1908. Also in *Boston M. and S. J.* **158:** 749–761, May 7, 1908. (With P. M. Ashburn)
83. Studies upon the amoebae in the intestine of man, *J. Infect. Dis.* **5:** 324–377, June 4, 1908.
84. Observations upon *Treponema pertenue* (Castellani) of yaws and the experimental production of the disease in monkeys, *Mil Surg.* **23:** 83–100, Aug., 200–220, Sept., 1908. (With P. M. Ashburn)
85. Classification of the malarial plasmodia, *Boston M. and S. J.* **160:** 677–679, May 27, 1909.
86. Observations of the United States Army Board for the Study of Tropical Diseases in the Philippine Islands upon *Filaria philippinensis*, *Entamoeba coli*, the etiology of dengue and *Treponema pertenue* and the experimental production of yaws in monkeys, *Tr. Soc. Trop. Med. & Hyg.* **2:** 172–201, 1909.
87. *Filaria* (*Microfilaria*) *philippinensis*, *J. Trop. Med. & Hyg.* **12:** 239–241, Aug. 16, 1909. (Edit. abstract, *N. Y. Med. J.* **85:** 1134, 1909)
88. The Army Medical Service, *Yale M. J.* **16:** 415–427, 1909–10.
89. The importance to the Army of diseases transmitted by mosquitos and methods for their prevention, *Mil Surg.* **26:** 292–308, March 1910.
90. The sexual forms of the malarial plasmodia occurring in the blood of man, *Arch. Int. Med.* **5:** 325–347, April 1910.
91. Further observations on *Paramoeba hominis*, an intestinal parasite of man, *Arch. Int. Med.* **6:** 1–11, July 1910.
92. Complement fixation with an antigenic crystal obtained from luetic liver, *J.A.M.A.* **55:** 1264–1267, Oct. 8, 1910.
93. Observations upon the Noguchi modification of the Wassermann complement fixation test in the diagnosis of lues in military service, *J. Exper. Med.* **12:** 726–745, Nov., 1910.
94. Is hemoglobinuric fever a manifestation of malaria or a disease *sui generis*? *Arch. Int. Med.* **7:** 56–84, Jan., 1911.
95. On the nature of the viruses of yellow fever, dengue and pappataci fever, *New York M. J.* **93:** 360–369, Feb. 25, 1911.
96. *Entamoeba tetragena* as a cause of dysentery in Philippine Islands, *Arch. Int. Med.* **7:** 362–366, March 1911.
97. Relation of certain bacteria to non-specific reactions with the complement fixation test for lues, *J. Exper. Med.* **13:** 521–530, May 1911.
98. Immediate effect on the complement fixation test for lues, of treatment with salvarsan (arseno-benzol); an analysis of 225 cases, *Arch. Int. Med.* **8:** 395–417, 1911.
99. Prophylaxis of malarial infection, *South. M. J.* **4:** 469–478, July, 1911.

100. The effect of the ingestion of alcohol on the result of the complement fixation test in syphilis, *J.A.M.A.* **57:** 474–476, Aug. 5, 1911.
101. Prophylaxis of malarial infection, *Mil Surg.* **29:** 302–317, Sept., 1911.
102. Further observations on the complement fixation test in lues, *J. Infect. Dis.* **9:** 213–228, Nov., 1911.
103. Malarial infections pp. 17–45; Hemoglobinuric (blackwater) fever. pp. 45–55; Trypanosomiasis pp. 56–60; Kala-azar, infantile splenic anemia and oriental sore (Leishmaniosis) pp. 61–66; Helminthiasis pp. 66–75. In Hare, H. A., Modern treatment. v. 2. Phil., Lea & Febiger, 1911.
104. Important factors in the prophylaxis of the malarial fevers, *South. M. J.* **5:** 50–57, Feb., 1912.
105. Observations upon the morphology of parasitic and cultural amebae, *J. Med.* **26:** 1–37, April 1912–13.
106. The parasitic amebae of man and their relation to disease, *New Orleans M. & S. J.* **65:** 1–17, June, 1912–13.
107. A study of complement fixation in syphilis with spirochaeta antigens, *J. Exper. Med.* **16:** 336–348, Sept. 1912. (With H. J. Nichols)
108. A national commission for the study and prevention of malaria, *Trans. 15th International Congress on Hygiene and Demography*, Wash., Govt. Print. Off., 1912.
109. The present status of our knowledge regarding the parasitic amebas, (Abstract) *Med. Rec.* **82:** 1053, Dec. 7, 1912.
110. The relation of parasitic amoebae to disease, *Am. J. Med. Sc.* **145:** 83–100, Jan., 1913.
111. The interpretation of the results of the Wassermann test, *J.A.M.A.* **60:** 565–569, Feb. 22, 1913.
112. Preliminary report of the Commission of the Southern Medical Association for the study and prevention of malaria, year ending Nov. 4, 1912, *South. M. J.* **6:** 219–226, April, 1913. (With G. E. Henderson *et al.*)
113. The identity of *Entamoeba histolytica* and *Entamoeba tetragena;* a preliminary note, *J.A.M.A.* **60:** 1353–1354, May 3, 1913.
114. The present status of our knowledge regarding the parasitic amoebae, *South. M. J.* **6:** 370–378, June 1913.
115. The identity of *Entamoeba histolytica* and *Entamoeba tetragena* with observations upon the morphology and life cycle of *Entamoeba histolytica*, *J. Infect. Dis.* **13:** 30–52, July 1913.
116. Results of antityphoid vaccination in foreign armies, War Dept. (U. S.) *S. G. O. Bull.* no. 2. pp. 15–22, Wash., 1913.
117. Is the importance of intestinal parasites in tropical pathology exaggerated? (Disc.) *Am. J. Trop. Dis.* (New Orleans) **1:** 170–171, Aug. 1913.
118. Classification of amoebae with special reference to the parasitic species, *Am. J. Trop. Dis.* **1:** 351–361, Nov., 1913.
119. Relation of parasitic amebae to disease, *Tr. XV Internat. Cong. Hyg. and Demog.* **2:** 150–168, 1913.
120. The prophylaxis and treatment of malarial infections, *Internat. Clinics*, Vol. 3, 23rd Series. J. B. Lippincott Co., Phila., 1913.
121. Variations in the strength of the Wassermann reaction in untreated syphilitic infections, *J.A.M.A.* **62:** 1232–1236, April 18, 1914.
122. The classification of amebas with observations on morphology and life cycle of *Entamoeba coli*, *Craigia hominis* and *Valkampfia lobospinosa*, *Arch. Int. Med.* **13:** 737–769, May 1914.
123. The differential diagnosis of the intestinal entamebas of man, *Arch. Int. Med.* **13:** 1917–1944, June, 1914.
124. Observations upon the morphology, life cycle and relation to disease of *Entamoeba histolytica*, *Am. J. Trop. Dis.* (N. O.) **2:** 169–184, Sept. 1914.

125. Observations upon the morphology, life cycle and relation to disease of *Entamoeba histolytica*, *Am. Soc. Trop. M.* **9:** 103–118; 197–205, 1914.
126. New varieties and species of plasmodia, *J. Parasitol.* **1:** 85–94, Dec. 1914.
127. The prophylaxis of malaria, with special reference to the military service, *Bull.* (6) *Office Surg. Gen. U. S. War Dept.*, 1914.
128. The malarial fevers. In Osler's Modern Medicine (McCrae) 2nd ed., Phila., Lea & Febiger, 1914.
129. The results and interpretation of the Wassermann test, *Am. J. Med. Sc.* **149:** 41–56, Jan., 1915.
130. Serological diagnosis of syphilis, *Med. Herald* **34:** 29–42, Feb., 1915.
131. The importance of tropical diseases to the American practitioner as illustrated by their occurrence in the U. S., *Am. J. Trop. Dis.* (N. O.) **3:** 5–16, July 1915.
132. Some practical points regarding the Wassermann test for syphilis, *J. Kansas M. Soc.* **15:** 359–365, 1915.
133. Observations upon the endamebae of the mouth, *Endamoeba gingivalis* (*buccalis*), *J. Infect. Dis.* **18:** 220–238, Feb., 1916.
134. Some practical points regarding the results and interpretations of the Wassermann test for syphilis, *Mil Surg.* **38:** 286–299, 1916. Also in *Am. J. Syphilis* **1:** 192–210, Jan., 1917.
135. Classification of parasitic amebae of man, *J. M. Research.* **35:** 425–442, Jan., 1917.
136. Occurrence of endamebic dysentery in troops serving in El Paso district from July 1916–December 1916, *Mil Surg.* **40:** 286–302 March; 423–434, April 1917.
137. Complement fixation test for tuberculosis, *J.A.M.A.* **68:** 773–776, March 10, 1917.
138. The parasitic amebae of man and their relation to disease, *New Orleans M. &. S. J.* **65:** 1–17, June 1917.
139. Triple typhoid vaccine, *J.A.M.A.* **69:** 1000–1003, Sept. 22, 1917.
140. Complement fixation test for syphilis, *Am. J. Syphilis* **1:** 802–820, Oct., 1917.
141. Classification of the parasitic amebae, *Proc. Am. Scient. Cong. Wash.* **10:** 536–546, 1917.
142. The Army Medical Museum, *Mil Surg.* **45:** 679–687, Dec., 1919.
143. Meliodosis Chapt. VIII-A, p. 202 (1); Oroya Fever and Verruga Peruana, Chapt. VIII-B, p. 202 (7); Black water fever, Chapt. XVII-B, pp. 484 (7); Dengue, Chapt. XVIII, p. 485; Seven day fever, Chapt. XXX-A, p. 730 (3); Rat-bite fever, Chapt. XXX-B, pp. 730 (9); Malaria, Chapt. XXXII, p. 739; Pappataci fever, Chapt. XXXII-A, pp. 798 (21); Amebiasis, Chapt. XXXIII, p. 799. In Oxford Medicine, edited by H. A. Christian, v. 5. New York, Oxford University Press, 1919.
144. Army medical museum and medical profession, *Modern Med.* **2:** 542–549, August 1920.
145. Etiology of dengue fever, *J.A.M.A.* **75:** 1171–1176, Oct. 30, 1920.
146. Classification and differential diagnosis of aestivo-autumnal plasmodia, *Am. J. Trop. Med.* **1:** 57–95, March 1921.
147. Experimental observations upon effect of cholesteremia on results of Wassermann test, *Am. J. Syphilis* **5:** 392–400, July 1921. (With W. C. Williams)
148. Unrecognized infections in production of carriers of pathogenic organisms, *J.A.M.A.* **77:** 827–833, Sept. 10, 1921.
149. Differential diagnosis of common intestinal amoebae of man, *Mil Surg.* **49:** 513–527, Nov., 1921.
150. Correspondence (on a paper by S. T. Darling entitled "The tertian characters of quotidian aestivo-autumnal fever"), *Am. J. Trop. Med.* **2:** 85–86, 1922.
151. Relative value of Sachs-Georgi and Wassermann reactions in diagnosis of syphilis, *J.A.M.A.* **79:** 1597–1601, Nov. 4, 1922. (With W. C. Williams)
152. The cultivation of *Endamoeba histolytica*, *Science* **64:** 406, Sept. 1926.
153. A simplified method for the cultivation of *Endamoeba histolytica*, *Am. J. Trop. Med.* **6:** 333–339, Sept. 1926.
154. Observations upon the cultivation of *Endamoeba histolytica*, *Am. J. Trop. Med.* **6:** 461–464, Nov., 1926.

155. The nuclear structure of *Dientamoeba fragilis*, *J. Parasitol.* **13**: 137–140, Dec., 1926.
156. Scientific apparatus and laboratory methods; cultivation of *Endamoeba histolytica*, *Science n.s.* **64**: 406, 1926.
157. Complement fixation in the diagnosis of infections with *Endamoeba histolytica* (Abstract), *J. Parasitol.* **14**: 126, Dec., 1927.
158. Observations upon complement fixation in the diagnosis of pulmonary tuberculosis, *Am. J. Med. Sc.* **150**: 781–791, Dec., 1927.
159. Observations upon the hemolytic, cytolytic and complement-binding properties of extracts of *Endamoeba histolytica*, *Am. J. Trop. Med.* **7**: 225–240, 1927. (Abridged in *Science* **65**: 620, June 24, 1927)
160. Henry James Nichols, 1877–1927, *Am. J. Trop. Med.* **7**: 353–355, Nov., 1927.
161. The symptomatology of infection with *Endamoeba histolytica* in carriers, *J.A.M.A.* **88**: 19–21, Jan. 1, 1927.
162. The value of cultural methods in surveys of parasitic amebae of man, *Am. J. Trop. Med.* **7**: 39–48, Jan., 1927. (With J. H. St. John)
163. Complement fixation in diagnosis of infections of *Endamoeba histolytica*, *Am. J. Trop. Med.* **8**: 29–37, Jan., 1928.
164. Symptomatology, diagnosis and treatment of carriers of *Endamoeba histolytica*, *J.A.M.A.* **90**: 1345–1349, April 28, 1928.
165. Observations upon complement fixation in infections with *Endamoeba histolytica*, *Proc. Nat. Acad. Sci.* **14**: 520–526, July, 1928.
166. Relation of officers of the Medical Corps to scientific medicine, *Mil. Surg.* **63**: 338–348, Sept., 1928.
167. Malaria, *Arch. Path.* **6**: 645–723, Oct., 1928.
168. Diagnosis and treatment of latent amoebic infection, *Internat. Clin.* **1**: 11–94, March 1929.
169. Comparison of practical value of Wassermann and Kahn tests in diagnosis of syphilis in military service, *Am. J. Syphilis* **13**: 206–215, April 1929.
170. The technique and results of a complement fixation test for the diagnosis of infections with *Endamoeba histolytica*, *Am. J. Trop. Med.* **9**: 277–296, Sept., 1929.
171. Directions for making the United States Army typhoid-paratyphoid "A" vaccine, Army Medical Center, Wash., Govt. Print. Off., 1929.
172. The diagnosis and treatment of amebiasis. In Billings & Forcheimer's "Therapeusis of Internal Diseases," Blumer Ed., Suppl. Vol., D. Appleton & Co., N. Y., 1929.
173. Henry J. Nichols, 1877–1927. *Rev. prat. d. mal. d. pays chauds* **9**: 454–456, Oct., 1929. (With M. Zeitoun)
174. Serological studies with *Endamoeba histolytica*. In "Problems and methods of research in protozoology," Hegner and Andrews, pp. 182–193, New York, Macmillan Co., 1930.
175. The diagnostic value of the complement fixation test in amebic infections, *J.A.M.A.* **95**: 10–13, July 5, 1930.
176. Cultivation of *Endamoeba histolytica*. In "Problems and methods of research in protozoology." Hegner and Andrews, pp. 171–181. New York, Macmillan Company, 1930.
177. El valor diagnóstico de la prueba de fijación del complemento en las infecciones amebianas, *Bol. Ejercito*, Habana **30**: 546–551, Jan.–Feb., 1931.
178. Complement fixation in the diagnosis of amebiasis, *Ann. Int. Med.* **5**: 170–177, Aug., 1931.
179. Failure to produce filterable forms of common pathogens in Kendall media, *Proc. Soc. Exper. Biol. & Med.* **29**: 661–662, Feb., 1932. (With F. M. Johns)
180. Observations on use of Kendall's medium in cultivation of *Endamoeba histolytica*, *Proc. Soc. Exper. Biol. & Med.* **29**: 663, Feb., 1932.
181. Observations upon complement fixation in experimental amebiasis in dogs, *Proc. Soc. Exper. Biol. & Med.* **30**: 270–272, 1932.
182. Amebiasis problem, *J.A.M.A.* **98**: 1615–1620, May 7, 1932.

183. The possible chemostatic effect of the salivary secretions of certain insects upon microfilariae, *Science* **75:** 561–562, May 27, 1932.
184. Pathology of amebiasis in carriers, *Am. J. Trop. Med.* **12:** 285–299, July, 1932.
185. Review of recent contributions on treatment of malaria, *South. M. J.* **25:** 541–544, Dec., 1932.
186. Diagnosis and treatment of intestinal amebiasis, *South. M. J.* **25:** 1207–1212, Dec., 1932.
187. The protozoal diseases. In Musser, J. H. Internal medicine, Chapt. VI, p. 241–286, Phila., Lea & Febiger, 1932.
188. In memoriam: Doctor William Sydney Thayer, 1864–1932, *Am. J. Trop. Med.* **13:** 141–142, March 1933.
189. Further observations on complement fixation test in diagnosis of amebiasis; analysis of results of test in 1,000 individuals, *J. Lab. & Clin. Med.* **18:** 873–881, June 1933.
190. Study of complement fixation in experimental amebiasis in dogs, *Am. J. Hyg.* **18:** 202–219, July 1933.
191. Nomenclature of *Plasmodium ovale* Stephens, 1922, *Am. J. Trop. Med.* **13:** 539–542, Sept., 1933.
192. Encephalitis lethargica and African sleeping sickness, *Internat. M. Dig.* **23:** 250–252, Oct., 1933.
193. Toxicity of emetine, *J.A.M.A.* **101:** 1984, Dec. 16, 1933.
194. Amebiasis, general review, *Internat. M. Dig.* **24:** 54–59, Jan., 1934.
195. Amebiasis and amebic dysentery, *Proc. Inst. Med.* Chicago **10:** 21–38, Feb. 15, 1934. Also in *Am. J. Dig. Dis. & Nutrition* **1:** 4–8, March 1934.
196. Ivy day address, *New Orleans M. & S. J.* **87:** 281–284, Nov., 1934.
197. Some research problems in malaria, *South. M. J.* **27:** 448–452, May 1934.
198. Results of recent research in treatment of malaria, *South. M. J.* **27:** 546–549, June 1934.
199. The epidemiology of amebiasis, *J.A.M.A.* **103:** 1061–1063, Oct. 6, 1934.
200. Clinical aspects of amebiasis, *New Orleans M. & S. J.* **86:** 609–613, March 1934.
201. Immunology of typhoid. Cyclopedia of Medicine, 1934.
202. The prevention and control of amebiasis, *New Orleans M. & S. J.* **88:** 209–219, Oct., 1935.
203. The diagnostic value of the complement fixation test in amebiasis (Spanish and French summaries), *Rev. Parasitol. Clin. y Lab.*, Habana, **2:** 305–314, May–June 1935.
204. Theobald Smith and insect transmission of disease, *Am. J. Trop. Med.* **15:** 407–414, July 1935.
205. Amebiasis. In McKinley, E. B. A Geography of Disease. Supp. to *American Journal of Tropical Medicine* **15:** 389–392, Sept., 1935.
206. Observations on antigens for complement fixation in amebiasis, *Proc. Soc. Exper. Biol. & Med.* **32:** 958–960, March 1935. (With L. C. Scott)
207. Observations upon the methods of transmission of amebiasis, *Am. J. Public Health* **25:** 1231–1236, Nov., 1935.
208. The malarial fevers, Chapt. V; Typhus fevers, Chapt. XXXII; Tropical diseases, Chapt. XXXVI. In Brennemann's Practice of Pediatrics v. 2. Hagerstown, Md., W. F. Prior Co., 1935.
209. Necessity for more accurate statistics regarding distribution and incidence of tropical disease in U. S., *Am. J. Trop. Med.* **16:** 15–24, Jan., 1936.
210. Some unsolved problems in parasitology of amebiasis, *J. Parasitol.* **22:** 1–13, Feb., 1936.
211. Diagnostic value of complement fixation test in amebiasis, *Rev. de parasitol. clin. y lab.* **2:** 305–314, May–June 1936.
212. Modern treatment of malaria, *New Orleans M. & S. J.* **89:** 697–699, June 1937.
213. Observations upon the practical value of the complement-fixation test in diagnosis of amebiasis, *Am. J. Pub. Health* **27:** 689–693, July 1937.

214. Importance of tropical medicine to Southern physicians, *J. M. Assn. Georgia* **26:** 364–367, July 1937.
215. Observations upon complement-fixation test in monkeys infected with *Endamoeba histolytica*, *Proc. Soc. Exper. Biol. & Med.* **37:** 671–673, Jan., 1938.
216. Suggested program of control of amebiasis by health departments, *Am. J. Pub. Health* **28:** 187–192, Feb., 1938.
217. Research upon malaria plasmodia, 1936–1937; etiology of malaria, *South. M. J.* **31:** 944–946, Aug., 1938.
218. Modern treatment of malarial infections, *Texas State J. Med.* **35:** 213–218, July 1939.
219. Nature and practical value of complement fixation test for amebiasis, *Texas State J. Med.* **35:** 554–560, Dec., 1939.
220. "Short-term" treatment of malarial infections with quinine, *Am. J. Trop. Med.* **20:** 239–248, March 1940.
221. Medicinal prophylaxis of amebiasis, *Am. J. Trop. Med.* **20:** 799–801, Nov., 1940.
222. The treatment of diseases caused by protozoa, Chapt. X–XI. In Reimann, H. A. ed. Treatment in general medicine, v. 1, pp. 211–287, Phil., F. A. Davis, 1940.
223. Protozoan infections, in Barr, D. P., ed. Modern medical therapy in general practice. v. 1, pp. 1828–1918. Baltimore, Williams & Wilkins, 1940.
224. Cyclical variation in incidence of malaria, Symposium on Human Malaria, pp. 131–134, 1941.
225. Dengue fever; Yaws (Frambesia); The typhus fevers; Infectious jaundice (Weil's disease). In Tice's Practice of Medicine, vol. 4, W. F. Prior Co., 1942.
226. Relapsing fevers, Chapt. XVIII, p. 259; Infectious jaundice, Chapt. XIX, p. 273; Rat-bite fever, Chapt. XX, p. 284; Seven-day fever, Chapt. XXI, p. 290; Dengue, Chapt. XXXIII, p. 421; Phlebotomus fever, Chapt. XXXIV, p. 432. In Bercovitz, Z. T., ed. Clinical tropical medicine. New York, P. B. Hoeber, 1944.

COLONEL CHARLES FRANKLIN CRAIG, M.C., U. S. ARMY

A Salute to the Chief

JAMES STEVENS SIMMONS

Brigadier General, U. S. Army (Retired), Dean and Professor of Public Health, Harvard School of Public Health

This issue of the American Journal of Tropical Medicine is dedicated to the high purpose of paying tribute to a great medical scientist, Colonel Charles Franklin Craig. As one of Colonel Craig's former students, as his devoted assistant during many stimulating years of teaching and research in the Department of Bacteriology at the Army Medical School, and as a comrade who has always cherished his friendship and advice, I welcome the opportunity to join with his many other admirers in this *Salute to my Chief*.

Colonel Craig is probably best known for his scientific contributions in the fields of parasitology and tropical medicine, and especially for his pioneer work on malaria, amebiasis, and dengue fever. His interests and experience, however, were much broader; in fact, his first love was bacteriology, and even before he entered the Medical Corps of the Regular Army he was recognized as a bacteriologist.

As I write these words, I have before me a photostatic copy of a biographical record form of the Association of Military Surgeons which was completed in longhand by Colonel Craig at the age of 29, while serving as "Pathologist and Bacteriologist" at the U. S. General Hospital, Presidio of San Francisco, 50 years ago. His familiar handwriting on this form fills me with mixed emotions. First, one of sadness that my good friend has finished his task and is gone; and then one of pride in the selfless manner with which he met all of his responsibilities.

As stated in this record, Charles Franklin Craig was born in Danbury, Connecticut on July 4, 1872, the son of William E. and Marion H. Craig. He received his premedical education at the public and high schools of Danbury and in the Bridgeport Business College. In 1894, at the age of 22, he graduated from the "Yale Medical Department" with the degree of M.D.

Young Dr. Craig spent his first four years as a civilian physician, but his activities during the period from 1894 to 1898 were indicative of his future interests as a military medical scientist. His first position was that of "Pathologist and Bacteriologist" at the New Haven Hospital, and later he occupied a similar post at the Danbury Hospital in his home town. During those four years he published ten scientific articles dealing with a wide variety of subjects. The first, entitled: "*A Study of the Microscopic Phenomena of Inflammation with special reference to diapedesis of the white blood corpuscle*" appeared in the Proceedings of the American Microscopic Society in 1894, the year of his graduation from Yale. The other nine were as follows: "*Umbilical hemorrhage*": Med. News, Phila., 1894; "*The Parasitic Origin of Carcinoma*": Proc. Conn. Med. Soc.,

Bridgeport, 1896; *"Recent Advances in our Knowledge Concerning the Malarial Organism"*: Med. Rec., N. Y., 1896; *"On Variations in the Morphology of the Bacillus Tuberculosis"*: Medicine, 1897; *"Notes on the Pathology and Bacteriology of Appendicitis"*: N. Eng. Med. Month., Danbury, Conn., 1897; *"Some Remarks Upon the Uric Acid Diathesis, and its Treatment"*: N. Eng. Med. Month., Danbury, Conn., 1897; *"The Transmission of Disease by the Mosquito"*: N. York Med. J., 1898; *"The Transmission of Disease by Certain Insects: Ticks, Bedbugs, Ants, etc."*: N. York Med. J., 1898; *"The Branched Form of the Bacillus Tuberculosis in Sputum"*: J. of Exp. Med., 1898.

These titles may not sound exciting today. However, when highlighted with historical perspective they point up the fact that our friend must have been stimulated at an early age by the exciting medical developments of the period in which he lived.

When the last of these articles was written, Dr. Charles Craig was only 25 years old. The first third of his life had been spent in the golden age of medical discoveries, when the researches of the pioneers in microbiology were rapidly uncovering the basic knowledge which later gave life to the sciences of bacteriology and protozoology. Eventually, this knowledge provided a firm foundation for the profession of medicine, and for its specialties of military preventive medicine and civilian public health.

BIRTHDAY OF PROMISE

On July 4, 1872—the day Charles Craig was born—our nation celebrated the 97th anniversary of its Declaration of Independence; the Civil War with its monstrous disease causalties was ended, General Grant was President of the United States, and the country was on the verge of the postwar financial panic of 1873. The American Medical Association had been in existence only 25 years and the American Public Health Association was organized in that year under its first President, Dr. Stephen Smith. However, the medical specialty, "Public Health", like its parent profession, "Medicine", was likewise in the process of birth. Both professions were still handicapped by the universal ignorance as to the etiology of most of the infectious diseases, and both were helpless when faced with the problem of treating or preventing these diseases. The underdeveloped status of medical microbiology at that time is indicated by the fact that it was not until a year later that Pasteur was elected to the French Academy of Medicine in recognition of his fundamental studies on fermentation, his so-called "Germ Theory", and his work on the diseases of silk worms; and it was several years before he did his brilliant work on the control of anthrax, chicken cholera, and rabies. The British surgeon, Lister, had only recently developed his so-called "antiseptic" operating technique, and the great German bacteriologist, Robert Koch, still had before him the productive years when he demonstrated the etiology of anthrax, tuberculosis, and cholera.

It is interesting to note that at the time of Colonel Craig's birth, no human being was aware of the causes of the three important diseases which were later to be studied most intensively by him. It was not until three years later, in 1875,

that Losch described an ameba, now accepted as *E. histolytica*, in the stools of a Russian suffering with dysentery in St. Petersburg. It was eight years later, in 1880, that Laveran, in North Africa, described the plasmodia of malaria. And it was not until a third of a century later, in 1907, that Colonel Craig himself, working with Ashburn in the Philippine Islands, discovered that dengue fever is caused by a fitrable virus.

CHILDHOOD OF INSPIRATION

Thus the birth of Charles Craig coincided with the birth of modern medicine. It was the beginning of a new era which was to free mankind from the fear of many diseases. He entered a world of adventure which offered many exciting opportunities for scientific investigation. By the time he was 15, the following disease organisms had been incriminated: *Borrelia recurrentis* (Obermeier 1873); *M. leprae* (Hansen 1874); *B. anthracis* (Koch 1876); *N. gonorrhoeae* (Neisser 1879); *E. typhosa* (Ebert 1880); *P. vivax and P. malariae* (Laveran 1880); *D. pneumoniae* (Pasteur-Sternberg 1880); *M. tuberculosis* (Koch 1882); *C. diptheriae* (Klebs 1883); *C. tetani* (Nicolaier 1884); *N. intracellularis* (Marchiafava and Celli 1884); *V. comma* (Koch 1884); *E. coli* (Escherich 1886) and *Br. melitensis* (Bruce 1887). During this same period, Patrick Manson in Hong Kong demonstrated the mosquito transmission of filariasis in 1877, and Carlos Finlay in Cuba reported in 1881 that he had transmitted yellow fever through stegomyia mosquitoes.

It was a long time, however, before these basic discoveries could be extended and applied effectively to the control of disease. The first volume of the American Journal of Public Health in 1873 was concerned largely with reports of yellow fever and of Asiatic cholera, which had occurred in about 200 towns in the Mississippi Valley. Pasteur's new "Germ Theory" was weighed against the prevalent theories concerning the causes of infections. Outbreaks of yellow fever occurred in Army posts in New York and Florida in 1874 and 1875, and this disease continued to invade the United States until after the work of Walter Reed at the beginning of the 20th century.

When Charles Craig was 16, in 1888, he became a clerk in the Danbury National Bank, but his destiny lay in the field of medicine. During the years that passed before he graduated from Yale in 1894, the great medical revolution continued to gain momentum. In 1889, Theobald Smith discovered the cause of Texas cattle fever and proved that it is transmitted by ticks. In 1890, von Behring discovered diptheria antitoxin. The next year, 1891, Robert Koch formed the Institute for Infectious Diseases in Berlin. In 1893, another great bacteriologist, George M. Sternberg, became Surgeon General of the U. S. Army; established the Army Medical School in Washington, and appointed Major Walter Reed as its first Professor of Bacteriology. Additional disease agents which were discovered, included: *H. influenzae* (Pfeiffer 1892); *C. welchii* (Welch and Nuttall 1892) and *P. pestis* (Yersin-Kitasato 1894). Also in 1894, Bruce went to Natal to start his work on Nagana, which led to the incrimination of the tsetse fly as vector of trypanosomiasis; and Patrick Manson inspired Ronald Ross to take up his work on the transmission of malaria.

During the four years of Dr. Craig's civilian practice, still other important events occurred. In 1896, *Cl. botulinum* was discovered by Ermenghem. In 1897, Sir Patrick Manson organized the London School of Tropical Medicine; and the same year, his disciple, Ronald Ross, announced the mosquito transmission of malaria, which was confirmed by Grassi. In 1898, Shiga discovered the dysentery bacillus. In April of that year, the United States declared war on Spain, and on August 3rd Charles Franklin Craig entered the Medical Corps of the U. S. Army.

A CAREER AS SOLDIER AND SCIENTIST

The year 1898 was an auspicious time for young Dr. Craig to join the Army. as it allowed him to continue to develop, along with his specialty. During the previous 26 years, the sciences of medical bacteriology and protozoology had also been born and they were rapidly gaining the stature of important disciplines of medicine. However, the filtrable virus diseases were still shrouded in mystery and no one had dreamed of the existence of the disease-producing rickettsiae. Epidemiology, the handmaiden of preventive medicine, was still ineffective and handicapped by the lack of specific knowledge about the hidden channels through which infections pass from man to man. Thus, when Charles Craig entered the Army he was not only inspired by the discoveries of the recent past, but was stimulated by the challenge of the still unknown causes of disease.

At that time the Army provided a favorable climate in which to develop his enthusiasm for medical research. Its Surgeon General, George M. Sternberg, was a great scientist who, five years earlier, had established the Army Medical School for postgraduate teaching and research, and had stimulated a broad program of investigation throughout the Army. General Sternberg wrote the first American text on Bacteriology; he and Pasteur independently discovered the Pneumococcus, and he was referred to by Robert Koch as "The Father of American Bacteriology". Another military scientist, Major Walter Reed, had been appointed Professor of Pathology and Bacteriology at the Army Medical School in 1893, and was now busy with investigations which preceded his epoch-making work on yellow fever. The young scientists, Frederick F. Russell, Richard P. Strong and Bailey K. Ashford had just entered the Medical Corps as First Lieutenants.

While the war with Spain was brief, the Army's experience with infectious diseases was important. It demonstrated the fact that the profession of medicine still lacked the knowledge required to control the common military plagues. American troops were afflicted by many diseases, including malaria and yellow fever, and typhoid fever was again a scourge even in the training camps at home. The incidence of typhoid reached the monstrous rate of 141 per thousand per annum.

Lieutenant Craig served as "Pathologist and Bacteriologist"—first, at the Sternberg General Hospital, Chickamauga Park, Georgia, where he had an opportunity to study the epidemiology of typhoid; later, at Fortress Munroe, Virginia and Camp Columbia, Havana, Cuba. In 1901, he occupied the same position at the U. S. General Hospital, Presidio of San Francisco.

These years at the turn of the century were productive ones both for the

Medical Corps and for Charles Craig. The Army's depressing wartime experience with disease was accepted as a challenge by Surgeon General Sternberg who redoubled his efforts to stimulate more research in order to develop better methods for the protection of troops. As a part of this expanded program, he established special research boards to study the diseases of our newly-acquired tropical possessions. In 1900, Major Walter Reed was sent as President of one of these boards to Cuba where he and his co-workers confirmed the claims of Carlos Finlay and showed that yellow fever can be transmitted by *Aedes aegypti*. Also, they proved for the first time that this disease is caused by a filtrable virus. In the same year another board was established for the study of tropical diseases in Manila, P. I., with Lieutenant Richard Pearson Strong as its first President. It was here that Richard Strong did his pioneer work on dysentery, cholera and plague, and it was on this research board that a succession of other officers, including Colonel Craig, worked on a wide variety of tropical diseases. At about the same time, Lieutenant Bailey K. Ashford in Puerto Rico began his studies of malignant Puerto Rican anemia, which showed that this disease was caused by massive hookworm infection. This led to the world wide campaign of The Rockefeller Foundation to control uncinariasis.

Lieutenant Craig's early work was done at the stations already mentioned, and continued in San Francisco until 1905. His first book, "The Estivo-Autumnal (Remittent) Malarial Fevers", was published by William Wood and Company in 1901. During the next few years, he had a rich opportunity to see many patients returned from the Philippines with tropical diseases through the port in San Francisco, and undoubtedly he was keenly interested in the reports made by by Lieutenant Strong and his associates on the Research Board in Manila. When he completed his service at the Presidio in 1905, he had already published 44 of the 226 scientific articles now included in his bibliography.

In the Philippines, Lieutenant Craig spent about 5 months of the year 1905 studying malaria in the field at Fort Stotzenberg; and on January 19, 1906 he was assigned with Captain Percy M. Ashburn as a member of the Research Board in Manila.

His studies there continued until 1907 and covered a wide range of diseases, including yaws, tsutsugamushi, filariasis and amebiasis. However, his most important work was on malaria and dengue fever. With Ashford, he proved that dengue is caused by a filtrable virus, and showed that in addition to its biological transmission by *A. aegypti*, the disease can be transferred mechanically through the interrupted feeding of Culex mosquitoes, an observation which was confirmed later by Simmons et al. (1931).

At the end of 1907, when Lieutenant Craig was relieved from duty in the Philippines, he was ordered to Washington, D. C. and assigned for four years as Professor of Bacteriology at the Army Medical School and Assistant Curator of the Army Medical Museum. In 1913, he had to leave Washington to comply with the so-called Manchu law, and was assigned as Commanding Officer of the Central Department Laboratory, Fort Leavenworth, Kansas. During the Mexican Border mobilization before World War I, he was transferred to Fort Bliss,

Texas, as Commanding Officer of the Southern Department Laboratory, No. 2; and in 1917 he was sent to Yale University to organize the enormous overseas training center known as the "Yale Army Laboratory School."

I first met Colonel Craig at Yale in 1918, two years after I entered the Regular Army. Following postgraduate work at the Army Medical School, I had served on the Mexican Border in charge of his former laboratory at Fort Bliss and then as Commanding Officer of the Southern Department Laboratories at Fort Sam Houston. From there, I had been ordered to report to Colonel Craig at the Yale Army Laboratory School, and with his assistance to organize an overseas stationary laboratory.

Following the war, Colonel Craig returned to Washington and for two years was Curator of the Army Medical Museum (1918–20), and then Professor of Bacteriology, Parasitology and Preventive Medicine at the Army Medical School, a post he had filled previously from 1909–13. This Professorship was first held by Major Walter Reed from 1893–1902, and was later occupied by a succession of men, including General F. F. Russell, Colonel Eugene R. Whitmore, Colonel Henry J. Nichols, and others. After several years as Director of Army Hospital Laboratories at Walter Reed in Washington, Tripler in Hawaii, and Fitzsimons in Denver, Colorado, I was privileged to follow in the footsteps of these distinguished military teachers and to occupy this same Chair of Bacteriology during eight of my thirty years of military service.

It was during these years in Washington that I had an opportunity to work closely with "The Chief," to fully appreciate his qualities of greatness, and to profit by his scientific advice. When I was first assigned to teach at the Army Medical School in 1924, Colonel Craig was Medical Inspector of the Hawaiian Department in Honolulu, where he wrote his sixth book, entitled: "A Manual of the Parasitic Protozoa of Man."

When Colonel Craig returned to the School in 1926, as Director of Preventive Medicine, I had already served two years as Professor of Bacteriology and as Assistant Director of Preventive Medicine. I continued in this work with him until 1928, when I was ordered to Manila as President of the Army Research Board. While I was overseas, "The Chief" continued to help me by mail with advice about our investigations of malaria and dengue fever. It was at his request that I was brought back to the School in 1930, to resume my work as Professor of Bacteriology and also Director of the Preventive Medicine Division under his direction as Commandant.

RETIREMENT TO CIVIL LIFE

After thirty years of military service, "The Chief" requested retirement in 1931, in order to accept the position of Professor and Head of the Department of Tropical Medicine at the Tulane Medical School. He held this civilian post until 1937, when he retired from Tulane and moved to San Antonio, Texas, where he continued his scientific work until his death on December 9, 1950.

Even after he took up his teaching and research at Tulane, Colonel Craig kept in touch with his "boys" in the Army, and we frequently called on him

for advice about important scientific and military activities. My own file of correspondence with "The Chief", which runs back to 1918, is filled with helpful letters relative to my work in various places, including: the Army Research Board in Panama (1934–36), Headquarters, First Service Command in Boston (1936–40), and during the wartime years when I had charge of the Preventive Medicine Service in the Surgeon General's Office (1940–46).

In this paper I have emphasized Colonel Craig's scientific contributions while a member of the Medical Corps of the Army, and have said little about his post-retirement work because he was primarily a military scientist and his most productive years were spent in the Army. During his 30 years of military service, he published more than 150 of his 226 scientific articles and 7 of his 10 important books. The last of these was the Third Edition of "Laboratory Methods of the U. S. Army", which he edited just before he retired from the Army. The Fourth Edition, which appeared in 1935, was dedicated to him by the editors with the following words:

> "Dedicated to Colonel Charles Franklin Craig, U. S. Army, Retired; soldier, scientist and author, Editor in Chief of the Third Edition of this Manual; and throughout his thirty years of achievement in the Medical Corps, a constructive force in the maintenance of its high ideals of scientific endeavor and service."

In addition to his military activities, Colonel Craig made a significant contribution as Editor of the American Journal of Tropical Medicine, a labor of love which he performed in a distinguished manner for 20 years, until it became necessary for him to resign in 1947, because of ill health. He was highly respected by his co-workers at Tulane and by thousands of scientific workers throughout the world. He received many honors, not only from the Army but from foreign governments, great universities, and distinguished scientific organizations, yet he remained all his life a simple, lovable searcher after truth. As I think back over the years in which I knew Colonel Craig, I am thankful for the privilege of his friendship.

Although he spent many years at the laboratory bench, "The Chief" found time for family life. He was married on July 7, 1893 to Lillian Osmun, and was devoted to her and to their two children, Marjorie Lillian and Edward Arthur. Ned became a General in the U. S. Marine Corps; and "The Chief"—like all of us—was very proud of the heroic record of his son, General Edward Craig, during the Korean war.

The Medical Corps is proud of Colonel Craig because he is a part of their rich tradition of scientific service to the Army and to the world. Like his great predecessors, George M. Sternberg and Walter Reed, and his distinguished contemporaries, Russell, Strong, Whitmore, Nichols, Vedder and Siler, Colonel Craig was a pioneer in military bacteriology and preventive medicine. Shoulder to shoulder with these fellow scientists, he spent his life in the search for new knowledge with which to attack the crippling diseases which have always afflicted both troops and civilians.

I am proud to join in this Salute to the Chief!

TROPICAL MEDICINE IN THE ARMED FORCES

WILLIAM S. STONE[1]

*Army Medical Service Graduate School**

In this presentation dealing with the Armed Forces' interest in tropical medicine, I must emphasize that the views presented are primarily those of the Army; however, the principles apply to the other services. The Armed Forces' interest in tropical medicine must always be primarily influenced by its actual or potential bearing on manpower, particularly during critical phases of military operations. The magnitude of such influence dictates the emphasis to be placed on the disease or other health factors concerned. For example, some of the Army's experience in World War II with some communicable diseases usually considered in the category of tropical disease is shown in table 1.

It can be seen that the dysenteries, particularly those bacterial in type, malaria, and virus hepatitis influenced manpower in a major way whereas diseases such as filariasis, schistosomiasis, and leishmaniasis were of very little significance.

What are the current military interests in tropical diseases? Before discussing these problems specifically, I would point out that the tropics are no longer remote, isolated areas, and many diseases that formerly were thought of as confined to tropical climes, are no longer limited to these areas but because of rapid transportation now in use, are apt to be encountered in any of the places inhabited by man.

The Armed Forces provide group environment for their members in contrast to the family environment to which most individuals are accustomed. Further, the Armed Forces are not limited to the protected, sanitated environment of the United States but their members may see duty in many foreign lands where sanitation is primitive and disease transmission favored. Frequently the Armed Forces are required to supervise the health of civil populations under their care as refugees or displaced persons. Under conditions of war the environment these populations must live under is extremely unfavorable for health, and resources are very limited for its improvement.

These wide fields of responsibility show the orientation of the Armed Forces' interest, and, bearing in mind that emphasis will always be placed on major causes of disability and manpower loss, the following list indicates in general the fields in which the Armed Forces are either conducting or sponsoring research:

1. Human
   a. Malaria
   b. Dysenteries
   c. Scrub typhus
   d. Schistosomiasis

[1]Colonel, MC, USA.

* Washington 12, D. C.

TABLE 1

Selected Categories Disease Cases, U. S. Army 1942–1945 Inclusive

	Total cases	Average cases per year
Encephalitis	508	127
Filariasis	4,036	1,009
Leishmaniasis	346	86
Poliomyelitis	1,326	331
Schistosomiasis	1,636	409
Coccidioidomycosis	2,894	723
Malaria	462,060	115,414
Acute respiratory diseases, including influenza	4,081,533	1,021,883
Diarrhea and dysentery	523,211	130,802
Infectious hepatitis	171,691	42,922
Venereal disease	1,071,429	267,857
	1944 *1945*	
Amebiasis	7,303 26,998	

TROPICAL MEDICINE RESEARCH

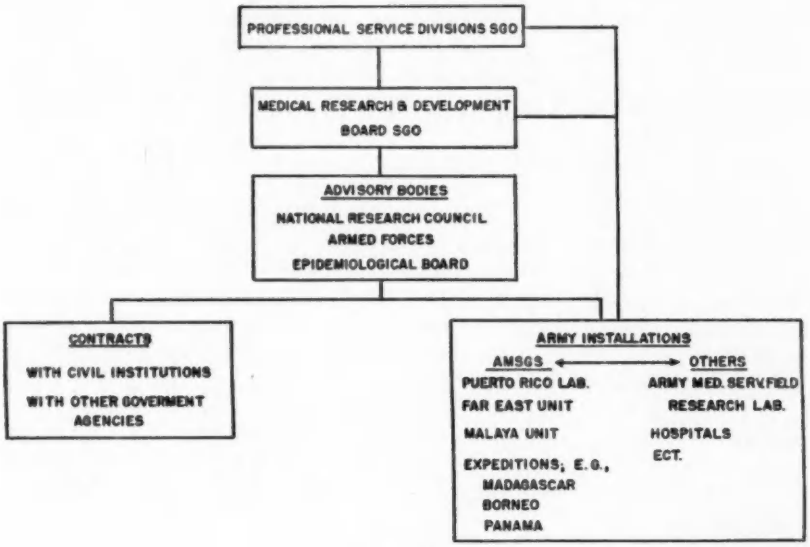


FIG. 1

e. Neurotropic viruses
f. Fungus and yeast infections
g. Bacterial infections of skin and chemical sensitization
h. Physiological and psychological requirements for homeostasis in tropical environments

i. Control of harassing agents—insects, heat rashes, land leeches, snakes, helminthic parasites, poisonous plants, poisonous foods
j. Dental materials and dental health
k. Other—leishmaniasis, leptospirosis, etc.

2. Animal

Prevention, control, and treatment of diseases in the tropics that affect Army animals

The approach to these problems is essentially basic in type with the utilization of applied research only where the problems of disease prevention, control, and therapy necessitate adaptation of existing knowledge to the military situations. For example, while testing of drugs and chemicals is done in clinical and sanitary work, we are much interested also in the mode of action of antibiotics and insecticides; the basic water and electrolyte requirements of troops under combat conditions, and the levels to be expected in wounded, with methods developed for their understanding and control. Basic studies on host-parasite relationships are under way or planned in the field of schistosomiasis both in regard to the life cycle in the mollusk and in man. Mere screening of chemicals just to find out if they have value as a molluscacide or as a therapeutic agent in man must be considered interim measures. Fundamental knowledge must be developed so that control and elimination of these parasites can be attained. The present advantage of effective residual acting insecticides has permitted a great step forward in the control of insect-borne diseases. As is evident, this advantage has not remained unchallenged. Most species of insects are now showing adaptive reactions to this unfavorable environment, and if we are to maintain and improve our advantage we must understand the mode of action of insecticides and meet each adaptation on the part of the insects by an effective countermeasure. Ways and means must be found to continue successfully to utilize interference reactions in some essential metabolic function of the insect vectors of disease.

Malaria is still being attacked by studies on prophylaxis, curative therapy, and destruction of infected vectors. Dysentery is responding well to sanitation and antibiotic therapy, but the method of transmission and the infective dose plus the influence of immunity on the clinical course of this disease are not understood.

The Army Medical Service's organization for the study of tropical disease is essentially as shown in figure 1.

It can be seen that this constitutes a careful fusing of military and civil talent not only at the advisory level but also at working levels. For example, there is currently a combined civil-military research group studying dysentery in Korea; antimalarial drugs are being studied in the United States and in Central America; and virus hepatitis investigations are under way in Japan, the United States, and Germany. These studies and the method of approach efficiently utilize civilian talent as well as military. The efforts exerted are for the common good as well as for the protection of the soldier. Great progress can be expected to continue if these efforts are understood, supported, and their results applied to health of the nation in peace as well as war.

RESEARCH ON DENGUE DURING WORLD WAR II[1]

ALBERT B. SABIN[2]

Army Epidemiological Board, Preventive Medicine Division, Office of the Surgeon General

STATUS OF PROBLEM PRIOR TO WORLD WAR II

Most of the basic and significant contributions to our knowledge of dengue prior to World War II were made by honored members of the medical department of the U. S. Army. Ashburn and Craig (1) provided the evidence for the viral etiology of the disease. Siler, Hall and Hitchens (2) clearly established the period of infectivity of dengue patients for *Aedes aegypti* mosquitoes, the period required for the development of the virus in these mosquitoes before they could transmit the infection, as well as the very long period during which these mosquitoes were capable of transmitting dengue. Simmons, St. John and Reynolds (3) established (a) the role of *Aedes albopictus* in the transmission of dengue, (b) the occurrence of inapparent infection in certain monkeys under experimental and possibly also natural conditions, thus suggesting the existence of a "jungle" type of dengue fever exclusive of the human cycle, (c) the persistence of immunity to the homologous strain of virus for 13 months in human volunteers residing in an endemic region, and (d) many of the properties of the virus. It is necessary to recall, however, that the latter investigators completed their studies in 1930, before most of the important, newer virological techniques and procedures had been developed. In 1934 Snijders, Postmus and Schüffner (4) reported some immunity experiments on human beings in Holland with two different strains of virus which left the subject of immunity to dengue in a rather unsettled state. In 1936, Shortt, Rao and Swaminath (5) reported the successful cultivation of dengue virus on the chorioallantoic membrane of chick embryos, but their conclusions were not based on tests on human beings. Otherwise, little or no work was done on dengue during the period of 1930 to 1940.

Thus, while a good deal of fundamental information about dengue was available at the beginning of World War II, it was also apparent that most of the elementary requirements which would permit one to carry out systematic studies with the virus of dengue fever were lacking. No strains of the virus were anywhere available; there was neither a suitable laboratory animal for experimental work nor an established method of *in vitro* cultivation, and almost nothing was known regarding some of the basic physical and biological properties of the virus.

[1] This article was prepared as part of the medical history program of the Army, and, with certain minor modifications, will appear in the forthcoming HISTORY OF PREVENTIVE MEDICINE, U. S. ARMY MEDICAL DEPARTMENT, WORLD WAR II. It is an honor and privilege to have this article published in a volume dedicated to Colonel Charles Franklin Craig, who, together with Ashburn, demonstrated the viral etiology of dengue and supplied a great deal of additional important fundamental information regarding this disease.

[2] Lieutenant Colonel, Medical Corps, Army of the United States. Present address: The Children's Hospital Research Foundation, University of Cincinnati College of Medicine, Cincinnati 29, Ohio.

PRIMARY OBJECTIVES OF RESEARCH OF MILITARY IMPORTANCE

From the point of view of military preventive medicine, there was a great need for an immunizing agent capable of protecting against dengue, preferably in a manner analogous to that achieved by the yellow fever vaccine. Another great need was for some practical, specific diagnostic test which would permit one to determine the precise role of dengue in the causation of the large number of "fevers of unknown origin" encountered in dengue endemic regions. It was clear that these needs could not be fulfilled until a great deal more was learned about the basic properties of the virus, the immunity which follows natural infection, and the immunological characteristics of strains from different parts of the world.

RESEARCH UNDER U. S. ARMY AUSPICES DURING WORLD WAR II

Organization of Dengue Research Unit[3]

Two basic principles for the organization of a dengue research unit were apparent from the beginning: (a) that no satisfactory work could be done without a constant, fairly large supply of human volunteers located within a relatively short distance from the laboratory, and (b) that it was better to carry on the work in a dengue-free area of the United States, bringing the viruses to the research unit, than to bring the research unit to an area where dengue was occurring in our troops. The unit was organized by this writer early in 1944, at a time when he was already engaged in studies on sandfly fever in human volunteers. The work was begun in the writer's laboratory at the Children's Hospital Research Foundation, University of Cincinnati College of Medicine, utilizing patients in need of fever therapy at the Longview State Hospital in Cincinnati, Ohio. Since the number of such patients was small, and most of those requiring fever therapy had already been used for tests with sandfly fever, the unit was transferred to New Jersey. The human volunteers consisted of white Americans who were serving sentences for civilian offences at the New Jersey State Prison at Trenton, New Jersey. The authorities of the state prison very kindly donated a hospital unit of seventeen beds which was mosquito-proofed and used for housing the volunteers during specified periods of experimentation. Two nurses (one day and one night) and a clinical laboratory technician assisted in the work of the hospital unit. The laboratory facilities were located at the Rockefeller Institute for Medical Research, near Princeton, New Jersey, approximately 15

[3] The great interest of Brig. Gen. James S. Simmons, MC, chief of preventive medicine, SGO, in the establishment of such a unit, the indefatigable help of Brig. Gen. S. Bayne-Jones, MC, deputy chief, preventive medicine, SGO and administrator of the Army Epidemiological Board, and the unfailing support of Dr. John R. Paul, director of the Commission on Neurotropic Viruses, were largely responsible for the creation and operation of the dengue research unit. The cooperation of the Longview State Hospital at Cincinnati, O. (Dr. Douglas Goldman), of The Rockefeller Institute at Princeton, N. J. (Dr. Carl Ten Broeck), and of the inmates and officials of the New Jersey State Prison at Trenton, N. J. was vital in the operations of this unit. The ASTP students of the University of Cincinnati College of Medicine, who volunteered for experimental inoculations in 1945, also rendered valuable service.

miles from the hospital unit in Trenton. Lt. (later Capt.) William L. Jahnes, Sn. C. was assigned to the unit as the entomological associate, and Lt. (later Capt.) R. Walter Schlesinger, M. C., joined the unit as a virological associate. Several civilian, technical aides were kindly made available by the Rockefeller Institute.

Recovery of Strains of Dengue Virus from Hawaii, New Guinea and India, and Criteria for Identification

During the period of 1944 to 1945, seven strains of dengue virus were recovered from Americans stationed in Hawaii (one strain), New Guinea (4 strains) and India (2 strains) by subinoculation of serum specimens into human volunteers in the U. S. A. The strains were recovered from serum specimens which were transported from overseas frozen in dry ice as well as from specimens which were merely refrigerated by ordinary ice. No sera were ever used in transmission tests until the patients from whom they were derived had recovered and it was clear that the clinical course was compatible with dengue. The first strain of virus was recovered in March 1944, when a pool of sera obtained 24 to 48 hours after onset from 6 patients with a diagnosis of dengue during an epidemic in Hawaii was subinoculated intracutaneously in 6 patients at Longview Hospital in Cincinnati, Ohio. All the subinoculated patients developed a febrile illness with rash and leukopenia, from which they promptly recovered. A large amount of serum obtained within 24 hours after onset of fever and stored partly in dry ice and partly in the lyophilized state served as a source of virus for many subsequent studies. Although the incubation period and clinical manifestations of the experimental disease were in most respects compatible with dengue, there was some concern about the occurrence of a rather marked, petechial eruption over the feet, ankles, legs and in some instances over the hands and wrists in 3 of the 6 patients toward the end of the febrile period or after defervescence. Since petechial hemorrhages had not previously been stressed in the clinical picture of dengue, it appeared necessary to determine whether or not a rickettsial agent might have been responsible. However, the convalescent sera of the 6 patients yielded negative results in Weil-Felix agglutination (OX-2 and OX-19) and rickettsial complement fixation tests, and inoculation of chick embryos into the yolk sac and of guinea pigs and mice with acute phase serum also failed to yield any evidence of rickettsiae. However, the identification of the virus as dengue was not considered established until transmission by *Aedes aegypti* mosquitoes, after the characteristic extrinsic incubation period, was accomplished. The continued occurrence of petechial hemorrhages in human volunteers after passage of the virus through mosquitoes and also after filtration through gradocol membranes with pores too small to permit the passage of rickettsiae finally eliminated the suspicion that the petechial hemorrhages might have been due to a rickettsial agent.

Sera from several types of febrile illness were collected in New Guinea between 30 April 1944 and 13 May 1944 and forwarded by Lt. Col. Cornelius B. Philip, Sn. C. in the fluid state refrigerated by ordinary ice. The sera, which were

suitable for study, were derived from essentially 3 types of cases:

a) Fevers of 2 to 3 days' duration followed by rapid recovery without rash, which were diagnosed as "Febricula" or "F. U. O."[4]

b) Fevers of 4 to 6 days' duration *without* rash which were regarded as dengue.

c) Fevers of 24 to 36 hours' duration, without rash, with leukocytosis of 10,000 to 19,000, diagnosed as F. U. O.

It was reported that the majority of cases in New Guinea were characterized by a short febrile course, and that very few exhibited rash or "classical saddleback" fever. The results of the subinoculation tests in human volunteers in the U. S. A. were as follows:

a) Four strains of virus were recovered which produced a febrile illness with rash, clinically compatible with dengue.

b) Two of the strains were obtained from the serum of patients who exhibited fever of approximately 2 days' duration, and the other two strains from the serum of patients who had fever of 4 days' duration or longer.

c) Although none of the 5 original patients in New Guinea, from whom these 4 strains of virus were recovered, was reported to have had a rash, all 8 volunteers inoculated either with the original serum or first passage serum exhibited a rash and febrile course, clinically compatible with dengue.

d) The experimental disease produced in human volunteers by the New Guinea strains was generally less severe than that produced by the Hawaii strain.

e) Cross resistance tests in human volunteers performed 4 to 8 weeks after the initial experimental attack served to identify the New Guinea viruses with the *Aedes aegypti* transmitted Hawaii dengue virus, although tests carried out after longer intervals subsequent to the original infection ultimately revealed that 3 of the 4 New Guinea strains were immunologically different from the Hawaii strain.

f) No virus was recovered from a pool of serum obtained 32 to 36 hours after onset from 2 patients who exhibited fever not exceeding 36 hours in duration associated with leukocytosis but without rash. The two volunteers used in this test showed no signs of illness and subsequently were proved to be susceptible to inoculation with 2 of the New Guinea strains of dengue. Accordingly, it was concluded that the brief, febrile illness with leukocytosis which was seen in New Guinea was most likely not a manifestation of dengue.

A variety of febrile illnesses, some with and others without rash, pleocytosis, or other changes in the cerebrospinal fluid, was occurring among U. S. Army personnel in India. It was not clear whether one or several etiological agents were involved, and whether or not the viruses of sandfly fever, dengue or one of the neurotropic viruses were among these agents. Col. Herman L. Blumgart, M. C., medical consultant in the India-Burma theater, obtained serum and cerebrospinal fluid from a number of such patients and the specimens were transported frozen in dry ice by air to the dengue laboratory in the U. S. The clinical

[4] Credit for stimulating interest in this group of patients is due to the many medical officers in the field, who in reporting these cases indicated their suspicion that the viruses of sandfly fever or dengue, or some other virus, might be responsible.

TABLE 1

Summary of Data on Isolation and Identification of Two Strains of Dengue Virus from "Fevers" Among U. S. Army Personnel in India

SOURCE	SPECIMENS TESTED	DATA ON ORIGINAL PATIENTS (DONORS)						RESULTS OF HUMAN TRANSMISSION TESTS								
									Isolation of Virus					Identification		
		Name	Duration of fever	Rash	Cerebrospinal fluid	Hospital Diagnosis	Days after onset specimen obtn.	Name of subject recipient	+ or 0	Inc. Per.	Fever		Leukopenia	Rash	Passage	Immunologic	Aedes aegyp. trans.
											Max.	Duration					
			days							*days*	*°F.*	*days*					
Calcutta 142nd. General Hospital	Serum	Kettrop	5 to 6	+	Protein 64 mg %	FUO	2	Bal	+	5	103.4	5	+ 2,200	0	+	+	+
								Schw	+	4	101.4	2	+ 3,350	0			
		Walker	4 to 5	0	Normal	Dengue	2	Bro	+	12	102.8	5.5	+ 4,150	+	+	+	+
		Ploof	4 to 5	+	Cells-0 Protein 50 mg %	Dengue	4	Alt	0	Subject subsequently proved susceptible to virus							
		Dillworth	4 to 5	+	Normal	Dengue	4	Mar	0	Subject subsequently proved susceptible to virus							
		Bennett	6 to 7	+	Normal	Dengue-like fever	5	Gos	0	Subject subsequently proved susceptible to virus							
	Cerebro spinal fluid	Kettrop	See Above				2	Cra	0								
		Dillworth + Bennett	See Above				4 5	Har	0								
New Delhi 100th Station	Serum	Henle	5	0	135 cells 70% lymph. Pandy +	None	3	Coo	0								

histories of some of the cases, particularly those occurring during the latter part of September 1945 in Calcutta, were compatible with dengue and it was considered worthwhile to attempt isolation of new strains of virus for immunological comparison with viruses already available from other parts of the world.

Serum from individual patients, rather than pools, were used for transmission studies to permit detection of multiple etiological agents and correlation of the clinical picture presented by the original patient with any virus that might be isolated. When pleocytosis was present, human transmission studies were postponed until it could be shown that neither the serum nor the cerebrospinal fluid contained an agent pathogenic for mice. The human transmission tests, summarized in Table 1, yielded two strains of virus. Both strains were recovered from the filtered serum (500 mμ gradocol membrane) of two individual patients, who became ill in Calcutta on 25 September and 27 September 1945 respectively. It is noteworthy that the cerebrospinal fluid yielded no virus, although in one instance it was obtained 2 days after onset at a time when the blood of the same patient (Kettrop) contained virus. No virus was recovered from the serum of the patient in New Delhi who exhibited a pleocytosis, and it may be pointed out that his clinical history was also not compatible with a diagnosis of dengue because he had a moderate leukocytosis instead of a leukopenia. Both strains of virus from India were identified as dengue on the basis of the following properties:

a) The clinical manifestations of the experimentally transmitted disease included skin lesions, which appeared after a suitable incubation period at the sites of intracutaneous injection of the serum, a self-limited fever after an incubation period that is usual for dengue, transitory leukopenia with the usual changes in the leukocytic formula, generalized macular or maculopapular rash and terminal petechial eruption.

b) Transmission of a clinically similar illness by *Aedes aegypti* mosquitoes after an extrinsic incubation period of 14 days following feeding on human beings infected with this virus.

c) Resistance of human beings recovered from infection with known dengue virus to inoculation with these new strains.

d) Human beings infected with the Calcutta strains developed neutralizing antibodies for the Hawaii mouse-adapted virus.

Properties of Dengue Viruses Determined by Studies on Human Volunteers

Minimal Infective Dose and Pathogenic Effects of Minimal Amounts of Virus.—Serum obtained from experimentally infected persons within the first few hours after onset of fever was stored in a dry ice chest and constituted the source of virus. The determination of the amount of virus present in such serum was desirable not only to permit quantitative work with this virus but also to ascertain whether minimal doses of virus produced a modified clinical syndrome and whether "subclinical" doses were immunogenic. Such infected serum, even after varying periods of storage in dry ice and repeated freezing and thawing, was found to contain one million human minimal infective doses (M. I. D.) per ml.,

when the dilutions were made in 10 per cent normal human serum-saline and inoculated intracutaneously. Ten M. I. D. injected intracutaneously produced as severe an infection as did one million M. I. D. However, one M. I. D. of virus produced different results in different individuals: (1) a typical unmodified attack resulted in 2 of 4 volunteers; (2) a short febrile illness without rash, followed by solid immunity, occurred in one of 4 volunteers, and (3) no evidence of infection, i.e., neither symptoms, fever nor leukocytic changes, followed by a partial immunity to reinfection in the fourth volunteer.

Cutaneous Lesions and the Local Sparing Phenomenon.—Intracutaneous injection of 0.1 to 0.2 ml. of human serum, containing 10 or more M. I. D. of dengue virus, was regularly followed after an interval of 3 to 5 days by local edema and erythema, 1 to 4 cm. in diameter. This skin lesion invariably appeared one or more days before onset of fever and, as a rule, disappeared before the occurrence of the generalized maculopapular or scarlatiniform eruption. However, when the generalized rash did appear, a striking sparing phenomenon was observed at the sites of the original skin lesions which stood out as blanched zones surrounded by the diffuse rash. This sparing was specific in that normal human serum or other irritants did not give rise to the same phenomenon. The specificity of the dengue skin lesion, and its dependence on local viral multiplication, were further demonstrated by the fact that no lesion occurred when a suitable number of M. I. D. of dengue virus were mixed with homologous convalescent serum prior to inoculation, or when the virus was inoculated into dengue convalescents. Injection of convalescent serum into an established maculopapular or scarlatiniform rash failed to cause blanching.

It was of interest to note that the local irritation and small papules resulting from the bites of dengue-infected *Aedes aegypti* mosquitoes could not be distinguished from those resulting from the bites of uninfected *Aedes aegypti* mosquitoes. However, when the generalized rash occurred, it was more marked at the sites originally bitten by the normal mosquitoes, while each papule resulting from the bite of an infected mosquito was surrounded by a blanched halo. Biopsies performed on the local skin lesions showed that the epithelium was not involved and no inclusion bodies were found. The chief abnormality was found in and about the small blood vessels and consisted of endothelial swelling, perivascular edema and infiltration with mononuclear cells.

Particle Size of the Virus.—The diameter of the virus as determined by filtration of highly infectious human serum through gradocol membranes was estimated at 12 to 25 millimicrons (mμ), because all the volunteers who received the filtrates from membranes with an average pore diameter (A. P. D.) of 75 mμ or greater developed typically severe dengue, while 2 volunteers who received the filtrate from the membrane with an A. P. D. of 50 mμ remained well. However, since the latter volunteers exhibited a partial immunity to reinoculation several months later, it is possible that approximately one M. I. D. of virus might have passed the 50 mμ membrane, and that the virus may actually be somewhat smaller than 17 to 25 mμ. The virus could be sedimented from human serum by centrifugation at 24,000 r.p.m. for 90 minutes in an 8-inch rotor of a vacuum ultracentrifuge. Examination with the electron microscope of preparations from

highly infectious human dengue serum, purified by differential centrifugation, revealed dumb-bell-shaped structures (700 mμ x 20–40 mμ), which were not found in similar preparations from normal human serum (studies carried out in association with Capt. R. W. Schlesinger, M. C. and Dr. Wendell M. Stanley of the Rockefeller Institute).

Cultivation in Various Media IN VITRO.—Although successful cultivation of dengue virus on the chorioallantoic membrane of the developing chick had been claimed, the claims were either not substantiated by tests on human beings,(5) or the human tests which were carried out yielded [in this writer's opinion] no conclusive evidence that the cultured material was dengue virus (6). Numerous attempts were made to propagate a variety of strains of the human, unmodified virus in embryonated eggs inoculated by various routes, or in tissue cultures containing minced chick embryo, minced mouse embryo, or human leukocytes. However, all of these attempts yielded negative results as judged by tests on human volunteers who failed to develop either disease or immunity. After the dengue virus was successfully adapted to propagation in mice, it proved possible to cultivate it in chick embryos.

Experimental Illness Following Infection with Dengue Virus by Scarification of Skin, Conjunctival or Nasal Instillation.—The investigation of the results of infection by "unnatural" routes was part of a search for some means of producing immunity without disease. Undiluted, infectious serum rubbed into the scarified skin produced unmodified dengue. Nasal instillation of one million or 100,000 M. I. D. (based on infectivity by intracutaneous route) resulted in a very mild or negligible illness with rash in 4 of 6 human volunteers, while 2 others suffered from a typical, unmodified attack. Nasal instillation of 10,000 M. I. D. produced neither disease nor immunity. Instillation of 200,000 M. I. D. into the conjunctival sac produced typical dengue in one volunteer, while 10,000 M. I. D. produced neither disease nor immunity in another. Mosquitoes feeding on a volunteer with the modified, negligible disease following nasal instillation of the virus, at the time the rash first appeared (in absence of fever), developed the capacity to transmit the unmodified disease. About 5 weeks after nasal instillation of the virus, 3 of the volunteers, who exhibited the mildest, symptomatic reaction, were exposed to the bites of dengue-infected mosquitoes and were found to be immune.

Effect of Atabrine and Penicillin.—The effect of atabrine on the course of dengue was investigated in order to determine whether or not this drug might have been responsible for the mild forms of dengue encountered in our troops in New Guinea. Three volunteers were given 0.1 gram of atabrine daily for a period of 12 days prior to infection by exposure to the bites of 22 to 24 *Aedes aegypti* mosquitoes. The same dose of atabrine was then continued throughout the incubation and febrile periods. All 3 volunteers, who were daily observed to swallow the drug, developed the disease without modification of the incubation period, clinical severity, rash or duration of fever. Penicillin administered at the onset of fever (25,000 units every 3 hours, day and night) had no effect on the course of the experimental disease.

Capacity of Certain American Mosquitoes to Transmit Dengue.—This investiga-

tion was carried out to determine to what extent mosquitoes prevalent in the U. S., other than *Aedes aegypti*, might be potential vectors. The feeding of any species on human volunteers, although occurring at the onset of fever, was always checked by allowing *Aedes aegypti* to feed simultaneously. The extrinsic incubation periods varied from 2 weeks to over a month. The following species of mosquitoes were found not to transmit the infection under conditions which permitted *Aedes aegypti* to act as an effective vector: *Aedes vexans*, *Aedes solicitans*, *Aedes taeniorhynchus*, *Aedes cantator*, *Anopheles punctipennis*, *Anopheles quadrimaculatus*, and *Culex pipiens*.

Preservation of Virus by Freezing and Lyophilization.—Dengue virus, in the form of human serum, has been found to be remarkably stable on storage in the frozen state in a dry ice chest, or in the lyophilized state in an ordinary refrigerator. Preparations have remained infective for a period of 5 years, the longest interval tested thus far (7).

Effect of Formalin and Ultraviolet Light on Infectivity and Immunogenic Capacity.—The virus in human serum or in mosquito suspensions was inactivated by ultraviolet light (an exposure of 0.47 sec. in the Oppenheimer-Levinson apparatus was sufficient) or by 0.05 per cent formalin, and when so inactivated, it failed to produce immunity in human volunteers.

Effect of Dengue Virus on Laboratory Animals

Infant mice and hamsters, newborn and adult guinea pigs, cotton rats, rabbits and rhesus monkeys inoculated intracerebrally or intra-abdominally, or both, with serum or whole blood of proved infectivity for human beings exhibited no clinical signs of infection. Tests with guinea pig brain tissue in a human volunteer revealed no evidence of inapparent infection in the guinea pig. On the other hand, subinoculation of serum obtained from rhesus monkeys 6 days after inoculation of human serum produced typical dengue in a human volunteer, thus confirming the occurrence of inapparent infection in rhesus monkeys. The ultimate adaptation of the virus in mice will be described subsequently.

Immunologic Studies on Human Volunteers

Immunity to Reinfection with Homologous and Heterologous Strains of Virus.—Human volunteers reinoculated with the same strain of virus proved to be completely immune for as long as 18 months after a single infection—the longest period tested thus far. These tests are especially significant because they were carried out on human beings residing in nondengue areas and there can be no question of the immunity having been reenforced by intercurrent, inapparent reinfection. The results of reinoculation with a heterologous strain were found to depend on the interval after the original attack. Active immunity to heterologous strains was, as a rule, demonstrable during the first 2 months after an attack. That this cross-immunity is most likely due to a group specific antigenic stimulus and not to nonspecific resistance resulting from a preceding febrile illness was confirmed by the fact that phlebotomus fever convalescents exhibited no such immunity to dengue. Reinfection with a different immunologic

type of dengue virus approximately 2 to 3 months after a primary attack had been found to give rise to malaise and slight fever for less than 24 hours, and mosquitoes which fed on such patients acquired the capacity to transmit the unmodified disease. Group immunity was still evident for as long as 9 months after the primary attack, since volunteers who were then shown to be resistant to the homologous type reacted with a rash-free, febrile illness of 2 to 3 days' duration upon inoculation with a heterologous type of dengue virus. These modified attacks, clinically not recognizable as dengue, were proved to be dengue by both mosquito and blood transmission tests. By this method of comparison it was found that 4 of the 7 human strains studied, i.e., the Hawaii, New Guinea "A", and 2 strains from India, belonged to one immunological type or group, while the other 3, all from New Guinea, belonged to another. Since more than one immunological type of dengue virus was thus found to be present in New Guinea at the same time, it is possible that reinfection with a heterologous type of virus may have been one of the causes for the many atypical febrile illnesses which were diagnosed as fever of unknown origin, but which were shown to be dengue by recovery of the virus.

Demonstration of Type-Specific Neutralizing Antibodies.—Previous attempts by other investigators to demonstrate a neutralizing antibody in convalescent dengue serum were unsuccessful. It became apparent that the previous lack of success was at least in part due to the fact that unknown quantities of virus were used in the tests. Thus, it proved possible to demonstrate that the serum of volunteers convalescent from infection with the Hawaiian strain of dengue virus was capable of neutralizing 1,000 but not 100,000 M. I. D. of virus. This means that when 0.9 ml. of convalescent serum was mixed with 0.1 ml. of acute dengue serum (with one million M. I. D. per 1 ml.) diluted 1:100, and the mixture, after *in vitro* incubation, was injected intracutaneously in 0.2 ml. amounts in a human volunteer, neither local lesions nor illness developed. Utilizing the skin surface of the arms, abdomen, and back, 20 to 40 different sera, including normal controls, could be tested simultaneously in a single volunteer, the presence or absence of neutralizing antibody in a given specimen being determined by the appearance or nonappearance of a local lesion. Neutralizing antibody against 1,000 M. I. D. of virus was demonstrated in the serum of Hawaii dengue convalescents obtained 1 week, 1, 2, 3, and 8 months after defervescence. The dermal neutralization tests which established the type-specificity of the antibody were performed with approximately ten minimal skin-lesion-producing doses. The results obtained in tests with 5 human strains of virus, shown in Table 2, indicate only 2 distinct immunologic types among them—the Hawaii and New Guinea "A" belonging to one type, and the New Guinea "B", "C", and "D" strains to another. Convalescent serum obtained during the first 2 months after an attack, when active immunity to heterologous types was readily demonstrable, contained only type-specific antibody in the dermal neutralization test.

Interference between 17-D Strain of Yellow Fever and Dengue Viruses.—The investigation on the relationship between the yellow fever and dengue viruses was undertaken to determine whether or not vaccination with the 17-D strain

of yellow fever virus can modify the clinical course and severity of dengue. This was carried out in search for an explanation for the mild form which dengue assumed in American troops in New Guinea. Thirty volunteers were used in this study and the following results were obtained:

1. When dengue virus, in amounts of 10 to 1 million human M. I. D., was inoculated *simultaneously* with, or *3 days after*, yellow fever vaccine, the onset of dengue was delayed for 3 to 6 days, and the resulting disease was milder and of shorter duration.

2. When the dengue virus was injected *1 week* after the yellow fever vaccine, the incubation period was unaffected, but the resulting disease was milder and of shorter duration.

3. When infection with dengue virus (either by the bites of infected mosquitoes or the inoculation of 10 human M. I. D. of infectious serum) was post-

TABLE 2

Differentiation between Strains of Dengue Virus by Dermal Neutralization Tests in Human Volunteers

STRAIN OF VIRUS	SKIN LESIONS RESULTING FROM MIXTURE WITH					
	Normal serum	Human convalescent serum				
		Hawaii	"N.G."A	"N.G."B	"N.G."C	"N.C."D
Hawaii	+	0	0	+	+	+
New Guinea "A"	+	0	0	+	+	+
New Guinea "B"	+	+	+	0	0	0
New Guinea "C"	+	+	+	0	0	0
New Guinea "D"	+	+?	+	0	0	0

Reproduced from Sabin, A. B.: The Dengue Group of Viruses and Its Family Relationships, Bact. Rev., **14**: 225, 1950.

poned for *5 weeks* after the yellow fever vaccine, neither the incubation period nor the duration or severity of the resulting dengue were modified.

It was concluded, therefore, that immunity to yellow fever, resulting from vaccination of human beings with the 17-D strain of yellow fever virus neither protects against nor modifies the disease resulting from infection with dengue virus. While a definite interference phenomenon was demonstrable during the period of propagation of the two viruses, and while the simultaneous injection of yellow fever vaccine and dengue virus resulted in a rather mild and modified form of dengue, it could not be regarded as a feasible method for the simultaneous immunization against both diseases.

Interference Between Dengue and Viscerotropic Yellow Fever Virus in Rhesus Monkeys and Mosquitoes

One of the reasons the problem of interference between these two viruses was pursued further is the peculiarity in the epidemiology of yellow fever that it has apparently spared many parts of the world (e.g., India, Indonesia, Australia,

etc.) where dengue has been endemic. The study on rhesus monkeys and mosquitoes was carried out in association with Dr. Max Theiler of the International Health Division of the Rockefeller Foundation. In the tests on human volunteers, the yellow fever virus (17-D) was the agent which produced the inapparent infection while dengue produced the clinically apparent disease. In the tests on rhesus monkeys, the dengue virus (highly infectious human serum injected intra-abdominally or intracerebrally) produced the inapparent infection while the viscerotropic yellow fever virus (Asibi strain) produced the clinically severe, fatal disease. When the dengue virus was injected in rhesus monkeys 2 or 3 days before the viscerotropic yellow fever virus, it interfered with the multiplication of the latter virus and 6 of 7 monkeys survived, while all 9 control monkeys, inoculated with yellow fever virus only, died. When the interval between the dengue and yellow fever inoculations was 4 to 7 days, there was still demonstrable interference with the multiplication of the yellow fever virus, but death of the monkeys, while postponed, was not prevented. When the yellow fever virus (100 M.L.D.) was injected one month after the dengue virus, 6 of 8 monkeys died in a manner which suggested that no significant cross immunity existed between the two viruses.

Since the *Aedes aegypti* mosquitoes serve as natural vectors for both the dengue and yellow fever viruses, and since available evidence indicated that mosquitoes remain infected for life, the possible occurrence of interference between these two viruses in mosquitoes was of special interest and possible epidemiologic significance. *Aedes aegypti* mosquitoes which first were proved to have become infected with dengue virus (by tests on human volunteers) were allowed to feed on monkeys infected with the highly virulent Asibi strain of yellow fever virus. After a suitable interval these mosquitoes were tested for their capacity to transmit yellow fever, and 2 of the 3 monkeys bitten by them died of yellow fever. However, tests in which extracts of individual mosquitoes were tested in mice suggested that some of the dengue-infected mosquitoes did not become infected with the yellow fever virus, while all the normal mosquitoes did. In view of the fact that in feeding on monkeys infected with the Asibi strain of yellow fever virus, each mosquito acquires about 10 million M.L.D. of virus, a degree of infection which may not obtain in nature, other experiments were performed in which normal and dengue-infected mosquitoes were allowed to feed on artificial mixtures containing varying amounts of yellow fever virus, and the multiplication of virus in them determined by tests in mice. These experiments strongly suggested interference with multiplication of smaller doses (about 10^5 LD_{50}, or less) of yellow fever in dengue-infected mosquitoes, and in another biting test in monkeys, the normal mosquitoes which ingested 10^5 LD_{50} of virus transmitted yellow fever, while a similar number of dengue-infected mosquitoes which fed on the same mixture did not. These results suggested the possibility that the introduction of yellow fever virus in a dengue-endemic area may find enough mosquitoes relatively refractory to the yellow fever virus to prevent the establishment of yellow fever in the same area.

Adaptation and Propagation of Dengue Virus in Mice

The following quotation from this writer's report to the Fourth International Congresses on Tropical Medicine and Malaria (8) summarizes the experiences encountered in the adaptation of dengue virus to mice:

"... the many similarities between the viruses of yellow fever and dengue and the available knowledge of the varying behavior of yellow fever virus in mice, were, in large measure, responsible for the persistence with which my associate, Dr. R. W. Schlesinger, and I continued the work on adaptation of dengue virus in mice in the face of many failures. Ultimately it appeared that in the primary adaptation of human dengue virus in mice, the important factors were the breed and age of the mice, the strain of virus, and the proportional concentration of virus and inhibitory factor apparently present in infectious human serum. The best results were obtained with the Hawaii virus, either concentrated by ultracentrifugation at 25,000 revolutions per minute for 90 minutes, or with highly infectious human serum diluted 1:100. The so-called Webster Swiss mice were better than other albino mice, and the "dba" mice bred at the Jackson Memorial Laboratory at Bar Harbor, Maine, appeared to be better than any of the albino and colored (C-57 black, C-57 brown) mice that were tested. Two-weeks-old or younger mice were needed for the initial passages, and it was not until the virus was thoroughly adapted after many serial passages in young mice, that older mice would succumb with regularity. The diagram in the accompanying chart (fig. 1) shows only that portion of the passage-tree which yielded successful consecutive passages and not the hundreds of mice which in the early passages exhibited nothing or yielded nothing on further passage. Only 10 to 20 per cent of the inoculated mice at first exhibited clinical signs of the infection(slight weakness of the extremities detectable only by special tests in some and distinct flaccid paralysis or encephalitic signs in others), and the incubation period was frequently 3 to 4 weeks. It took 15 passages before 100 per cent of the mice inoculated with a 10 per cent brain and cord suspension succumbed, and the incubation period was reduced to 9 to 14 days. At this stage the 50-per cent morbidity and mortality endpoint in mice did not exceed 10^{-2}, but continued serial passages in young mice gradually increased the titer and shortened the incubation period until now, after more than 80 such passages, the intracerebral titer for the 0.03 cubic centimeters dose in mice is $10^{-5.5}$, and the incubation period for the highest concentration is approximately 6 days. We could not be certain that this virus in mice was indeed dengue virus, until, after appropriate preliminary tests in laboratory animals, the early passage material was inoculated in human volunteers and produced in them solid immunity to unmodified human dengue virus. Similarly, we know that the virus of higher potency which is now being passaged in mice is still dengue virus, because it is neutralized specifically by the human convalescent sera and by the sera of rhesus monkeys and chimpanzees inoculated with human virus that has never been through mice. This mouse-adapted dengue virus produces neither apparent nor inapparent infection in cotton rats, hamsters, guinea pigs, or rabbits ..."

Effect on Human Volunteers of Mouse-Propagated Dengue Virus at Different Stages of Adaptation

Extracts of the brain and spinal cord of paralyzed mice, derived from the first 6 consecutive passages in mice, upon inoculation in human volunteers produced clinical manifestations of varying severity — relatively mild in some and fully severe and unmodified in others (9). Local skin lesions appeared at the sites of intracutaneous injection after an incubation period of 5 to 7 days, and

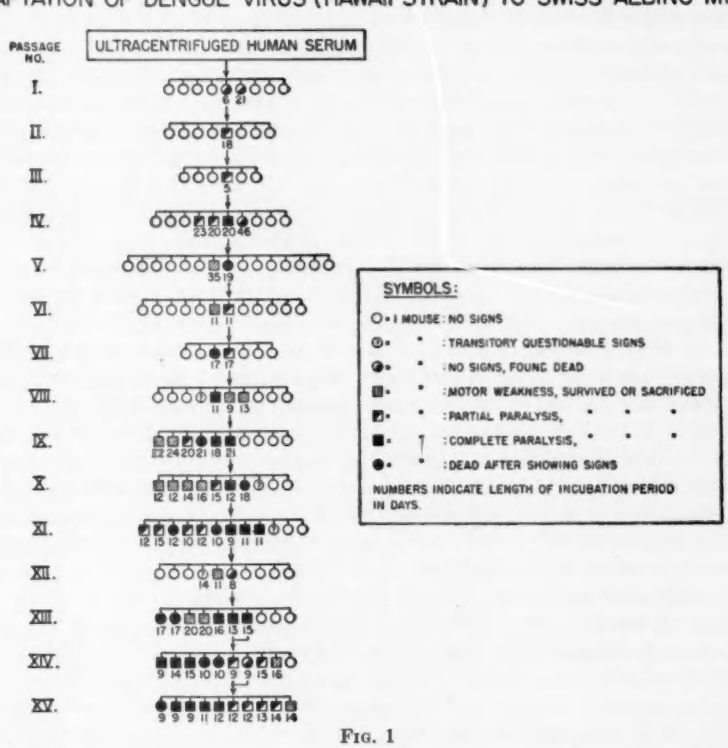


FIG. 1

marked generalized maculo-papular and ultimately petechial eruptions appeared in all. Leukopenia with the qualitative and quantitative changes in the leukocytic formula usually seen in dengue were also present. Blood serum obtained from the above volunteers at the onset of the generalized eruption produced typical dengue in another volunteer.

Beginning with the seventh passage in mice, however, the virus had lost its capacity to produce the severe illness and protracted fever, characteristic of the unmodified disease in human beings, but retained its capacity to produce a rash and solid immunity to the unmodified virus. Fever either did not occur,

or was low-grade, lasting 24 hours, or less. Blood taken from such persons at the time the rash first appeared produced only the same type of modified reaction in other volunteers. Immunity to infection with the unmodified virus was already present 12 days after inoculation, the shortest interval tested.

Use of Mouse-Adapted Dengue Virus as a Vaccine

The results of tests on 9 human volunteers inoculated with the seventh to the tenth mouse-passage virus indicated that the virus had undergone sufficient attenuation to permit its consideration for use as a vaccine against dengue. Accordingly, two vaccines were prepared from fifteenth and nineteenth mouse-passage virus and tested both in medical student volunteers of the Army Specialized Training Program and in schizophrenic patients. The fifteenth mouse-passage vaccine, consisting of a centrifuged 1:10 mouse-brain extract in 10 per cent human serum-saline solution, was tested in 16 individuals in doses of 0.5 ml. of the 1:100, 1:1,000 and 1:10,000 dilutions. While the titer of this virus in mice was only 1:100 for the 0.03 ml. dose, all inoculated persons (including those receiving 0.5 ml. of the 1:10,000 dilution) developed a maculo-papular eruption of varying extent, while systemic symptoms were either absent, negligible or very mild. Twenty-one to 38 days after vaccination, all were found to be immune upon exposure to *Aedes aegypti* mosquitoes of proved infectivity. Thus, it became apparent that the extract from a single mouse brain and cord, preserved in the frozen state, had enough antigen in it to immunize at least 10,000 people. When some of the same fifteenth mouse-passage vaccine was lyophilized together with yellow fever vaccine and tested in 10 students (the inoculum contained 1:100 or 1:1,000 dengue and the standard dose of yellow fever vaccine), 5 of 7 students subsequently exposed to dengue-infected mosquitoes were not immune. It was not clear whether the chick embryo yellow fever vaccine did not constitute a suitable protective agent for the lyophilization of the diluted, modified dengue virus, or whether the proportions of the two viruses in the inoculum were such that the multiplication of the smaller dose of modified dengue virus was suppressed by the larger dose of yellow fever virus.

Since it was desirable to get away from human serum as a constituent of the vaccine, crystalline bovine albumin was selected as the protein to be used for both extraction and lyophilization in the tests with the nineteenth mouse-passage vaccine. A relatively large amount of vaccine (enough for more than 50,000 men) was prepared both as a model for production on a still larger scale and for the purpose of having enough available for a field trial if the preliminary human tests were satisfactory. With the aid of the facilities of the laboratories of the International Health Division of the Rockefeller Foundation, the vaccine was lyophilized in 2 forms—as 10 per cent and as 1 per cent extract of mouse brain and cord in 10 per cent crystalline bovine albumin. Simultaneous titrations in mice of the frozen and lyophilized 10 per cent vaccines revealed that the lyophilized material had only $\frac{1}{10}$ as much infective virus as the frozen preparation, i.e., that 90 per cent of the infectivity was lost on lyophilization. However, tests on 15 persons revealed that the 10 per cent lyophilized vaccine produced

the same type of reactions (i.e., predominantly rash) as the fifteenth mouse-passage frozen vaccine, except that in the 1:10,000 dilution, the lyophilized preparation produced rash in only 1 of 3 individuals, and immunity to a large dose of unmodified dengue virus (probably as much as 1 million M. I. D.) in 2 of the 3 individuals. The 1 per cent lyophilized vaccine was apparently ineffective (probably more deterioration on lyophilization) since neither rash nor immunity resulted from the 1:1,000 or the 1:10,000 doses.

Each vial of the 10 per cent lyophilized vaccine contained 1 ml. of the centrifuged extract of mouse brain and cord, and was to be reconstituted in 20 ml. of saline. Thus 0.1 ml. of this diluted material (the projected dose for subcutaneous injection) contained the mouse brain and cord extract as well as the crystalline bovalbumin in a final dilution of 1:200, and one vial would have supplied enough vaccine for approximately 200 men. This vaccine was to be used in a field trial among troops who were being transferred from Europe to the Pacific, but the war fortunately came to an end before this eventuality occurred.

Infectivity of AEDES AEGYPTI *Mosquitoes Feeding on Human Beings Inoculated with Mouse-Adapted Dengue Virus*

This investigation was prompted by two practical questions: (a) is it possible that the virus might revert to its original, unmodified virulence after passage through mosquitoes and (b) could *Aedes aegypti* mosquitoes transmit the modified infection after feeding on vaccinated individuals at certain periods after inoculation? The following results were obtained: (a) *Aedes aegypti* mosquitoes which fed on the human volunteer inoculated with the second and third mouse-passage virus (i.e., before fixed modification had occurred) during the period of low-grade fever produced unmodified dengue in another volunteer after an extrinsic incubation period of 22 days, but not after 15 days, even though 40 mosquitoes fully engorged. (b) *Aedes aegypti* mosquitoes which fed on people inoculated with the tenth mouse-passage virus acquired the capacity to transmit the modified infection, i.e., the bitten volunteer developed only the rash and leukopenia without fever or other significant symptoms. (c) Extensive tests carried out on students, inoculated with the fifteenth mouse-passage virus, indicated that several lots of *Aedes aegypti* mosquitoes, which had fed daily on the experimental subjects for 14 days after vaccination, were unable to transmit the infection even after prolonged extrinsic incubation periods of 29 to 33 days. With 22 to 42 mosquitoes engorging in the tests on each volunteer, there was no rash or other clinical evidence of infection in any of the 14 men who were exposed in these tests.

Cultivation of Mouse-Adapted Dengue Virus in Chick Embryos

No success was achieved in the cultivation of dengue virus in chick embryos until the virus had undergone approximately 16 consecutive passages in mice. Attempts to propagate the mouse-adapted virus, using passages IV, V and XIII, in fluid or plasma clot cultures containing mouse embryo brain tissue were unsuccessful in that no virus pathogenic for mice was demonstrable after one, two

or three passages. When the 13th passage mouse-adapted virus was used for inoculation of 6 or 10 day old chick embryos which were subsequently incubated for 8 or 4 days, respectively, at 37° C, no mouse-pathogenic virus was demonstrable after one to four serial passages. The results were the same when the inocula were introduced into the yolk sac or embryo, allantoic or amniotic sacs. When the 13th passage mouse-adapted virus was inoculated directly into the brain of 10 day old embryos which were subsequently incubated for 7 days at 37° C, mouse-pathogenic virus was demonstrable in the chick embryo brain tissue in the first passage, but not in the second. Repetition of this procedure with 16th passage mouse-adapted virus yielded negative results even in the first passage. When the 16th or 18th passage mouse-adapted virus was used for inoculation (toward the embryo) of 5 day old chick embryos which were subsequently incubated for 8 days at 37° C, mouse-pathogenic virus was demonstrable in whole embryo extract of the first passage in 3 separate series. In only one of these series, however, was virus demonstrable in the second passage, but even in this series it was no longer present in the 3rd passage when the incubation was at 37° C. It was found, however, that incubation at 35° C was more suitable for the serial propagation of the virus, so that it has been possible to demonstrate appreciable amounts of virus in the 3rd passage of at least 2 series. There was some indication that 4 or 5 days of incubation was not as good as 8 days, and that while the virus was present in the amniotic membrane as well as in the whole embryo, little or no virus was found in the amniotic fluid.

It finally proved possible to obtain continuous cultivation of the virus in embryonated eggs when 5-day embryos were used for inoculation and a period of from 8 to 10 days at 35° C for incubation. Tests in 4 human beings with extracts of the 3rd passage whole chick embryo or amniotic membrane revealed that the virus had remained in its modified form, producing the characteristic macular or maculopapular eruption after an incubation period of 9 to 11 days, without fever (except for elevation of 1°F for 24 hours in one subject) or other clinical manifestations.[5]

Serological Tests with Mouse-Adapted Dengue Virus

After the Hawaii dengue virus became sufficiently adapted to mice to yield an intracerebral titer of 10^{-3} to 10^{-4}, it was possible to develop a mouse test for the detection of neutralizing antibody. At that stage no neutralization of the virus could be demonstrated without preliminary incubation of the mixtures at 37° C for 2 hours, or if the serum was heated at 56° C for 30 minutes. Neutralization tests performed under optimal conditions (i.e., with sera transported and stored in dry ice, and serum-virus mixtures incubated at 37° C for 2 hours) on sera from human volunteers infected with various strains of virus and from individuals with histories of naturally acquired infections in various parts of the world yielded considerable interesting information. Thus, it was established that the neutralizing antibody was type-specific and appeared as early as 1 week

[5] Further work on cultivation of dengue virus in eggs has since been published by R. W. Schlesinger, *Am. J. Hyg.*, 1950, *51:* 248; *Proc. Soc. Exp. Biol. and Med.*, 1951, *76:* 817.

after onset of illness and persisted for at least 2 years (more recently, at least 4 years) in individuals residing in nondengue areas. Neutralization tests on sera from people with a diagnosis of dengue during the Hawaii epidemic of 1943–1944, or the Japanese epidemics of 1944–1945, revealed that the Hawaii type of virus was probably predominant in those outbreaks, while similar tests on the sera of Americans who had the disease on Guam in 1944–1945, and from Americans and Panamanians in the Panama Canal Zone indicated that another type or types of dengue were probably more prevalent there.

TESTS FOR DENGUE IN THE PHILIPPINES AND OKINAWA DURING WORLD WAR II

Large numbers of cases of a febrile illness (or febrile illnesses) clinically similar to dengue, with the important exception that there was no rash, occurred in 1945 in the Philippine Islands and on Okinawa. In view of the success that was encountered in the recovery of several strains of dengue virus from clinically atypical cases in New Guinea, repeated attempts were made to demonstrate the presence of dengue virus in patients with this atypical illness in the Philippines and Okinawa. The serological tests for dengue were not yet adequately developed at the time, and reliance had to be placed on the inoculation of serum, obtained within 24 to 48 hours after onset of fever, into human beings in the U. S. Three pools of sera derived from 11 patients in Leyte, P. I. (February–March 1945) were subinoculated into 6 human beings in the U. S., and 2 sera from 2 patients on Mindoro, P. I. (April 1945) were subinoculated into 2 human beings in the U. S., but all the recipients remained well. Although the donor patients were selected because they remained free of jaundice, there was, nevertheless, a great deal of hepatitis in those regions at the time. It is noteworthy, therefore, that the recipients were observed for many months but did not develop jaundice. The sera of 5 patients from Okinawa (June–August 1945) were similarly inoculated into 4 human beings in the U. S. with completely negative results.[6]

DENGUE IN PANAMA CANAL ZONE DURING WORLD WAR II

An investigation into the occurrence of dengue in Panama during World War II was carried out in January and February 1946. It was found that in 1941 and early 1942, at a time when a great deal of new activity was in progress in the Canal Zone and when there was an influx not only of military personnel but also of laborers from adjacent countries, there was a considerable outbreak in Americans of a disease with clinical manifestations entirely compatible with a diagnosis of dengue. However, the diagnosis of dengue was not made and practically all of these cases were found in the files of the Gorgas Hospital under the diagnoses of "nasopharyngitis," "nasopharyngitis with rash," or occasionally

[6] Recent work (Gauld, R. *et al.*, Leptospiral meningitis: report of outbreak among American troops on Okinawa, J. A. M. A., in press) suggests that these illnesses might have been due to leptospira; the negative human transmission tests could be accounted for by the fact that the sera were frozen in dry ice and leptospira are not well preserved in the frozen state.

"X-Y-Z" fever. However, Major L. McCarty Fairchild, (1945) M. C., of the Gorgas Hospital reported a selected series of these cases as "Dengue-like Fever in Panama." (10) My own analysis of the charts of these patients indicated that clinically they represented classical forms of dengue. It is of interest that *Aedes aegypti* breeding in Ancon, Balboa and Panama City were sufficiently high (indexes of 10 to 17 per cent) to justify warnings regarding intensified mosquito control. It is furthermore of interest that during this same period the "great majority" (variously estimated as 80 per cent) of the native Panamanian population in Panama City was attacked by a disease which was diagnosed for the most part as measles, German measles, or glandular fever. However, some of the Panamanian physicians (11), suspected dengue at the time and my own examination of many of the clinical records revealed syndromes clinically characteristic of dengue. There were apparently no further outbreaks after 1942, but sporadic cases probably continued to occur since I observed four clinically characteristic cases in Americans during my visit early in 1946. Neutralization tests in mice yielded positive results with 3 of 8 convalescent sera from the 1941–1942 outbreak, and 1 of 6 sera from recent convalescents. It would appear that a type of dengue virus, immunologically distinct from the Hawaiian strain, may be prevalent in Panama. This investigation also suggested the possibility (a) that the interior of Panama may be an endemic focus of dengue, and (b) that the *Haemagogus* mosquitoes and monkeys in the jungles of Panama should be investigated as a possible source of "jungle dengue fever."

DENGUE IN JAPAN DURING WORLD WAR II

According to an inquiry carried out by the writer in Japan in 1946, it would appear that approximately 2 million cases of dengue may have occurred in the port cities of Nagasaki, Kure, Sasebo, Kobe and Osaka between 1942 and 1945 (8). Dengue was said to have been unknown in Japan before 1942, and the epidemics were correlated with two facts: (a) These cities were the ports of entry for people from Shanghai, Singapore, and the Malay States where dengue was prevalent at the time, and (b) the water shortage and the later bombing led to the storage of water in all sorts of containers which became the breeding grounds for *Aedes albopictus*. The city of Osaka with a population of about 2 million had about 5,000 cases in 1942; 3,000 to 4,000 cases in 1943; 400,000 to 600,000 cases in 1944, and an unknown number in 1945, when, because of the air raids, the population dispersed to the surrounding villages. The situation was said to be similar in other port cities. Examination of the clinical charts of cases which occurred among the hospital personnel in Osaka revealed the classical clinical picture of dengue, and blood obtained from many of these patients in 1946 neutralized the mouse-adapted dengue virus.

Many Japanese investigators carried out experimental studies on dengue during the war, and many strains of virus were reported to have been adapted to a variety of experimental animals. Some of these animal-adapted viruses were no longer available in 1946. However, of 5 strains of "dengue" virus which were submitted by several investigators for comparative study, only the 3 mouse-

adapted strains recovered by Doctors S. Hotta and R. Kimura of Kyoto turned out to possess the properties of dengue virus; the other 2 "dengue" viruses submitted by others turned out to be Rift Valley Fever in one instance and fixed rabies virus in another. The neutralization tests performed with the Japanese convalescent sera indicated that the Hawaii type of virus was either predominant in, or exclusively responsible for, the epidemics in Japan.

SUMMARY

Research on dengue in the U. S. during World War II provided the following new information of special interest to military preventive medicine:

1. Proof of the existence of multiple immunological types of dengue.
2. The long persistence of immunity to homologous types of virus under conditions precluding reenforcement of immunity by subclinical reinfection.
3. The modifications of the clinical manifestations of the disease which result from reinfection with a heterologous type of virus at various periods after the primary attack.
4. The demonstration that in areas (e.g., New Guinea) where more than one imunological type of virus is present, fevers of unknown origin, clinically not recognizable as dengue, are actually caused by the dengue viruses.
5. The demonstration that type-specific immunity to dengue is associated with neutralizing antibodies for the virus, which can be used for diagnostic and epidemiologic survey purposes.
6. The propagation of dengue virus in mice with the resulting appearance of a mutant or variant strain which could be used for active immunization.

In addition to the discoveries listed above, a great deal more was learned about the basic properties of the dengue viruses. Thus was dengue research brought from the field into the laboratory and further progress has been made possible by work on experimental animals instead of on human volunteers.

REFERENCES

In future issues of this Journal, references will be cited by date and not by number.

1. Ashburn, P. M., and Craig, C. F., Experimental investigations regarding the etiology of dengue fever, *J. Infect. Dis.* **4:** 440–475, 1907.
2. Siler, J. F., Hall, M. W., and Hitchens, A. P., Dengue, its history, epidemiology, mechanism of transmission, etiology, clinical manifestations, immunity and prevention, *Philippine J. Sci.* **29:** 1–304, 1926.
3. Simmons, J. S., St. John, J. H., and Reynolds, F. H. K., Experimental studies of dengue, *Philippine J. Sci.* **44:** 1–247, 1931 (Monograph 29 of the Bureau of Science, Manila).
4. Snijders, E. P., Postmus, S., and Schüffner, W. A. P., On protective power of yellow fever sera and dengue sera against yellow fever virus, *Am. J. Trop. Med.* **14:** 519, 1934.
5. Shortt, H. E., Rao, R. S., and Swaminath, C. S., Cultivation of the viruses of sandfly fever and dengue fever on the chorio-allantoic membrane of the chick embryo, *Indian J. Med. Res.* **23:** 865, 1936.
6. Kimura, R., Higashi, N., and Akazawa, H., Über die Züchtung des Denguevirus auf der Chorioallantoismembran von Hühnereiern, *Japanese J. Med. Sc., VI. Bact. and Parasitol.* **2:** 241, 1944.

7. SABIN, A. B.: The Dengue Group of Viruses and Its Family Relationships, *Bact. Reviews*, **14**: 225, 1950.
8. SABIN, A. B., Recent advances in phlebotomus and dengue fevers, *Proc. Fourth Internat. Cong. Trop. Med. and Mal.*, 1948, 520–525.
9. SABIN, A. B., AND SCHLESINGER, R. W., Production of immunity to dengue with virus modified by propagation in mice, *Science* **101**: 640, 1945.
10. FAIRCHILD, L. McC., Dengue-like fever on Isthmus of Panama, *Am. J. Trop. Med.* **25**: 397, 1945.
11. ROGNONI, M., Epidemic disease in Panama (Editorial), *Boletín Asociación Medica Nacional Panama* **3**: 7, 1941.

PROPAGATION OF DENGUE VIRUS STRAINS IN UNWEANED MICE[1]

GORDON MEIKLEJOHN, BEATRICE ENGLAND, AND EDWIN H. LENNETTE

Viral and Rickettsial Disease Laboratory, California State Department of Public Health[2]

Several strains of dengue virus have been adapted to mice by serial intracerebral passage (Kimura and Hotta[3] (1); Sabin and Schlesinger (2) and Sabin (3)). Certain of these strains, viz., the Hawaiian, isolated by Sabin and Schlesinger (2), and the Mochizuki and Kin-A (1) are lethal, even in relatively high dilutions, for young adult mice. With other strains, notably the New Guinea C and D (3), considerable difficulty has been encountered in adapting the virus to adult mice and, even after repeated passages, the virus content of infected mouse brains has been so low as to hamper the performance of certain types of serologic and biologic procedures (4).

It was shown in this laboratory in 1947 that, in work with the Hawaiian strain, unweaned mice were superior to adult mice in a number of ways (5). The virus concentration in the mouse brains was higher, and, in titrations, deaths occurred with greater uniformity and within a shorter period of time. Satisfactory antigens for complement-fixation tests could be more readily prepared (6). More recently, it has been possible to compare the behavior of 4 additional strains of dengue virus in unweaned and in adult mice. Observations made during the adaptation of two New Guinea strains to mice, the results of parallel titrations of all 5 strains in unweaned and adult mice, and data bearing on the immunologic relationships of these strains are presented in this paper.

MATERIALS AND METHODS

Virus strains.—The Hawaiian strain, originally adapted to mice by Sabin and Schlesinger (2), was obtained in its 35th passage from the Naval Medical Research Institute. It was then carried through 5 additional intracerebral passages in young adult mice, and subsequently through 63 intracerebral passages in unweaned mice of 4–6 days of age. The Mochizuki and Kin-A strains were obtained from Japan through the courtesy of Dr. M. Kitaoka of the National Institutes of Health of Japan. The strains New Guinea C and New Guinea D were isolated in, and adapted to, unweaned mice (2–4 days of age) in this laboratory; the sources of these strains were sera from patients ill with dengue.

[1] The initial phases of this study were carried out under the auspices of Naval Medical Research Unit No. 2.

[2] 1392 University Avenue, Berkeley 2, California.

[3] The paper of Kimura and Hotta reported that mice inoculated with serum from patients with dengue had become sick and that a viral agent had been recovered; no evidence was presented to show that the agent recovered was dengue virus and not something else. Actually, the identity of the Japanese viruses was first established in Sabin's laboratory by comparison of the Japanese strains with his own strains of dengue virus which had been identified in a variety of ways, including the crucial test of transmission by *Aedes aegypti* (see references 7 and 8).

These sera, which were made available through the courtesy of Dr. Albert Sabin, had been stored for more than 5 years, first in the frozen, and later in the lyophilized, state.

Preparation of viral suspensions.—Two to 4-day old mice were inoculated by the intracerebral route; when moribund, the animals were killed and the brains removed for passage. The brains were ground in a mortar without abrasive and made to a 20 per cent suspension by incremental addition of 0.85 per cent saline containing 30 per cent of normal rabbit serum (serum-saline inactivated at 60°C. for 30 minutes). This suspension was spun at 3,000 r.p.m. for 30 minutes in the angle head of a refrigerated (4°C.) centrifuge. The supernatant fluid was distributed in 0.8 ml. amounts into small ampoules, which were flame-sealed and stored on dry ice.

Infectivity titrations of viral suspensions were carried out under conditions essentially identical with those employed in the neutralization tests. Serial 10-fold dilutions of the 20 per cent stock virus suspension were made in 30 per cent normal rabbit serum saline. To an aliquot of each virus dilution was added an equal volume of inactivated normal rabbit serum; the serum-virus mixtures were incubated for 4 hours at 4°C. and then inoculated into mice. Mice 2 to 6 days of age received 0.01 ml. intracerebrally, and adult mice of 21 to 28 days of age received 0.03 ml. intracerebrally. Mice dying before the third day or after the twenty-first day after inoculation were excluded from the mortality data used in computing the 50 per cent mortality endpoint.

Neutralization tests.—These were performed according to the procedure described just above for titration. Mice of 3 to 5 days of age were used. Immune sera, both monkey and human, were inactivated, prior to use, at 56°C. for 20 minutes; no attempt was made to supply, by addition of fresh, unheated normal serum, the "accessory factor" reported by Sabin (7). Normal monkey or human sera were included in the tests as controls and as a basis for estimation of the neutralization indices.

Monkey immune sera.—Monkeys (*Macaca mulatta*) were inoculated intracerebrally with a single dose of 2 ml. of a 1×10^{-1} suspension of infected mouse brain. The neutralization indices of the sera were low, however, and because they failed to rise during an interval of 2 months after the inoculation, the animals were given a second injection of virus, consisting of 5 ml. intravenously of a 1×10^{-2} dilution of mouse brain virus. Serum specimens collected 2 to 3 weeks after the intravenous inoculation were used in the tests described below; the specimens were stored at -10°C. in a compressor-operated refrigerator.

RESULTS

Adaptation of New Guinea C and D strains to mice.—The human serum containing the New Guinea C strain was passed serially both in unweaned mice (2 to 4 days old) and in young adult mice (21 to 28 days old).

In the unweaned mice, during the first 4 passages no animals died and none became paralyzed within two weeks following inoculation. Blind passage was carried out at 7 to 11 day intervals. In the 5th passage 2 animals appeared

ataxic and a 3rd had sustained tremors on the 9th day; these 3 animals were sacrificed and their brains were used for passage. In the 6th passage all animals were dead by the 8th day, and thereafter, through the 15th passage, all mice died, usually between the 6th and 8th days. The LD_{50} endpoint obtained in a titration of 9th passage material was $10^{-6.5}$.

In adult mice, 9 serial blind passages were carried out. The passages were made at 7 to 12 day intervals. Deaths occurred irregularly, and there was no good indication that adaptation of the virus was occurring. The brains of a few of the adult mice that died were subinoculated into 3–4 day old mice; these animals survived without symptoms of infection, and it was concluded that the deaths among the adult mice were due to non-specific causes.

TABLE 1

Titration of five strains of dengue virus by the intracerebral route in unweaned and in adult mice

STRAIN	MOUSE PASSAGE	AGE OF MICE	MORTALITY RATIO OF MICE INOCULATED WITH VIRUS DILUTION OF							LD_{50} ENDPOINT
			10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	
Hawaiian	104*	3–5 days			6/6	12/12	8/12	5/12	1/6	$10^{-6.9}$
		3–4 weeks	7/8	8/8	8/8	5/8	3/8	2/8	0/8	$10^{-5.9}$
Mochizuki	93*	3–5 days	6/6	6/6	6/6	6/6	5/6	3/6	0/6	$10^{-6.9}$
		3–4 weeks	5/6	4/6	5/5	5/6	5/6	3/6	2/6	$10^{-6.5}$
Kin-A	55*	3 days	12/12	12/12	12/12	5/9	5/12	1/9	0/8	$10^{-5.6}$
		3–4 weeks	6/6	5/5	5/5	3/5	1/5	0/6	0/6	$10^{-5.3}$
New Guinea C	9†	3 days	6/6	6/6	2/2	6/6	7/9	1/7	1/3	$10^{-6.5}$
		3–4 weeks	3/6	3/6	1/5	3/5	2/6	4/6	1/6	‡
New Guinea D	13†	3 days	6/6	6/6	7/7	8/8	7/7	0/7	1/3	$10^{-6.5}$
		3–4 weeks	4/6	1/6	1/6	3/6	1/6	0/6	1/6	‡

* Isolated, and passaged, in adult mice

† Isolated, and passaged, in unweaned mice (2–4 days of age).

‡ Calculation not made because of irregularity in mortality ratios.

With the serum containing the New Guinea D strain, adaptation was attempted only in unweaned mice. The procedure used and sequence of events observed followed those described for the New Guinea C strain, with the exception that 10 passages rather than 6 were required before adaptation had reached the point at which the inoculated mice uniformly succumbed to infection. The LD_{50} endpoint obtained in a titration of 13th passage material was $10^{-6.5}$.

Attempts to isolate a virus by a similar procedure from serum of a patient infected with the New Guinea B strain were unsuccessful. None of the mice inoculated during the course of the 32 blind passages which were made died during the period of observation.

Titration of 5 strains of dengue virus in unweaned and in adult mice.—In Table 1 data are presented for comparison of the results of titrations of the 5 virus strains in unweaned and in adult animals. The most striking difference was

apparent with the New Guinea strains. With these strains, deaths in the unweaned animals occurred uniformly and the LD_{50} endpoints were comparable to those obtained with strains (Hawaiian, Mochizuki, and Kin-A) which had been carried through many passages in adult mice. On the other hand, while deaths occurred among adult animals inoculated with high dilutions of the New Guinea strains, there were survivors at all dilutions between 10^{-6} and 10^{-2}.

With strains Mochizuki and Kin-A, the difference between adult and unweaned mice was minor in terms of lethality, for both strains killed adult mice in a pattern which was regular and comparable to that observed in unweaned mice.

The pattern obtained with the Hawaiian strain fell between those of the Japanese and the New Guinea strains; in adult mice, deaths occurred somewhat irregularly and the LD_{50} was somewhat lower than in unweaned mice.

TABLE 2

Average survival time of unweaned and of adult mice inoculated with different strains of dengue virus

STRAIN	AGE OF MICE	AVERAGE SURVIVAL TIME, IN DAYS, OF MICE INOCULATED WITH VIRUS DILUTION OF					
		10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Hawaiian	3 days	—*	—	8.7	9.5	11.8	16.8
	3–4 weeks	11.2	13.0	13.5	18.5	19.1	19.5
Mochizuki	3 days	7.8	8.5	8.8	9.2	11.3	17.8
	3–4 weeks	11.3	14.2	10.8	15.0	13.7	16.9
Kin-A	3 days	9.1	10.1	10.4	15.9	18.0	20.2
	3–4 weeks	10.5	11.4	12.6	17.2	20.2	21.0
New Guinea C	3 days	8.2	9.2	9.5	11.0	15.2	20.7
	3–4 weeks	16.2	15.7	18.6	16.4	19.3	16.3
New Guinea D	3 days	10.5	11.7	11.9	12.5	14.1	21.0
	3–4 weeks	15.5	20.2	20.2	18.8	20.8	21.0

*— = not done.

Average survival times (AST) were calculated on the basis of a 21-day observation period, and are presented in Table 2. For any given strain, the AST at each virus dilution was shorter in unweaned mice than in adult mice. In general, when deaths occurred among the unweaned mice, they did so before the 14th day, even at dilutions approaching the endpoint; in adult mice, specific deaths occurred as late as the 28th day.

The differences between the AST's in unweaned and in adult mice were greatest with the New Guinea strains, as would be expected, since these, as mentioned above, killed only erratically over the range of virus dilutions representing the titrations. The erratic behavior of these strains in adult mice also gave rise to the disparity shown in Table 2 between the AST and the concentration of virus inoculated, viz., in adult mice the AST did not increase progressively with a decrease of virus in the inoculum, as it did in the case of the Hawaiian and Kin-A strains.

Cross-neutralization tests.—The Hawaiian, Mochizuki and New Guinea C and D strains were examined for immunologic relationships in a series of cross-neutralization tests. Immune sera to the several strains were prepared in monkeys as described above. Also included in the tests was a monkey immune serum (M-934) prepared against the New Guinea C strain by Dr. Sabin; the monkey from which this serum came had been inoculated with human serum virus that had never been passaged through mice.

At this point, a few words about the neutralization tests are indicated as concerns the immune sera. Since the immune sera against the Hawaiian and Mochizuki strains were prepared by inoculation of monkeys with strains whose identity as mouse passage strains of dengue virus is established, there is little question as to the specificity of these 2 sera. The antisera to the New Guinea C and D strains, however, were prepared, as will be recalled, by inoculation of monkeys with infectious mouse brains representing a mouse passage line of

TABLE 3

Results of cross-neutralization tests in 3–5 day old mice with 4 strains of dengue virus

SERUM FROM MONKEY IMMUNIZED WITH	MONKEY NUMBER	DATE OF BLEEDING	VIRUS EMPLOYED IN NEUTRALIZATION TESTS			
			Hawaiian	Mochizuki	New Guinea C	New Guinea D
Strain			*Neutralization indices*			
Hawaiian	M-13	12/5/50	760	125	25	5
Mochizuki	M-25	12/14/50	1,300	2,500	10	8
New Guinea C	M-12	12/14/50	15	5	>15,000	>20,000
	M-934*	10/28/47	5		>20,000	
New Guinea D	M-24	1/16/51	25	2		>25,000

* Serum obtained from Dr. A. B. Sabin; monkey inoculated with human virus that had never been passaged in mice.

an agent contained in, and presumably derived from, the original starting material, human serum. To prove that the mouse passage virus is dengue virus, and not a latent virus of the mouse activated by the passage procedure, requires that the antisera be shown to contain specific antibodies to known dengue virus, or that the passage virus is neutralized by known dengue immune serum. It will be observed in Table 3 that the passage viruses representing the New Guinea C and D lines were neutralized by the serum of monkey 12, immunized with mouse brains from the New Guinea C line, and hence the 2 agents may be considered as similar, or identical. Since the agent representing the New Guinea C line was neutralized by the serum of monkey 934, immunized with known dengue virus, it would appear that the agent under passage as New Guinea C is actually a dengue virus, and that the serum of monkey 12 consequently contains antibodies to the dengue virus. In addition, since the serum of monkey 12, containing dengue antibodies, also neutralized the agent being passaged as New Guinea D, this agent would likewise appear to be a dengue virus.

Further evidence of the identity of these viruses was supplied by Dr. Sabin,

to whom brain material from the mouse passage lines of the New Guinea C and D strains was sent. The results of his neutralization tests with the New Guinea C mouse passage virus are presented in Table 4, for which we are indebted to Dr. Sabin, who prepared it and kindly permitted us to include it in this report. As shown in Table 4, Dr. Sabin found that the New Guinea C mouse-adapted virus was neutralized by convalescent sera from humans re-

TABLE 4

*Neutralization of New Guinea "C" virus (18th mouse passage) by various human and rhesus dengue convalescent sera**

SERUM SPECIMEN	NO. OF MICE INFECTED† AT INDICATED DILUTIONS OF VIRUS						ID_{50}	NEUTRALIZATION INDEX
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}		
Control—normal rabbit	—	5/5	5/5	5/5	2/5	0/5	6.8	—
Hawaii—human conv.—*Crown*—3 months	5/5	5/5	1/5	0/5	0/5	—	4.6	160
N. G. "B"—human conv.—*Vuono*—2 months	3/5	0/5	0/2	0/5	0/5	—	3.2–?	4,000 + ?
N. G. "C"—human conv.—*Clark*—29 day	2/5	0/5	0/5	0/5	0/5	—	2.8–?	10,000 + ?
N. G. "D"—human conv.—*Barba*—2 months	1/5	1/5	0/5	0/5	0/5	—	2.7–?	13,000 + ?
Hawaii—rhesus 945—37 day	3/5	2/5	4/5	1/4	0/5	—	4.6	160
N. G. "B"—rhesus 569—4 week	0/4	0/5	0/5	0/5	0/5	—	2.5–?	20,000 + ?
N. G. "C"—rhesus 934—38 day	1/4	0/5	0/5	0/4	0/5	—	2.7–?	13,000 + ?
N. G. "D"—rhesus 570—6 week	1/4	0/4	0/5	0/5	0/4	—	2.7–?	13,000 + ?

* This test was performed in Dr. Sabin's laboratory, and we are indebted to him for preparation of this table and for permission to include it in this report. The neutralization test was carried out with sera that had been stored in the frozen state and were not heated prior to use; the serum-virus mixtures were incubated for 2 hours at 37°C. prior to inoculation. Four-week-old mice, inoculated by the intracerebral route, were used.

† Only 2 mice of all those listed failed to die, but both exhibited definite paralysis.

The rhesus antisera were prepared by inoculation of human virus that had never been passaged in mice.

The Hawaii rhesus antiserum had a homologous neutralization index of 50,000. The N. G. "B", "C", and "D" rhesus antisera completely failed to neutralize the Hawaii virus.

covered from infection with the New Guinea B, C, or D viruses, and by the sera of monkeys immunized with human strains of these viruses.

Returning now to the cross-neutralization tests performed in our laboratory, the results presented in Table 3 indicate that the 4 strains tested fall into 2 immunologic groups. In the first group are the Hawaiian and Mochizuki strains, which appear to be closely related to each other. In the second group are the New Guinea C and D strains. These results agree with those previously reported by Sabin (7), who found that there are at least 2 immunologic types of dengue virus, and with Dr. Sabin's data presented in Table 4, which also point to the existence of immunologically different types.

COMMENT

The data presented here indicate that in work with the dengue virus, the use of unweaned mice is associated with advantages not always obtainable with the use of adult animals. Among these may be mentioned (1) the ease with which the virus is adapted to growth in the very young animal; (2) the higher concentrations attained by the virus in the brain; (3) the shorter survival time; and (4) the more uniform and consistent response of the immature animals to the lethal properties of the virus.

The Japanese strains (Mochizuki and Kin-A) can be handled, and studied, fairly satisfactorily in adult mice, and adequate work can be done with the Hawaiian strain in mice 14 to 16 days of age (5, 7) or older (7). Presumably, this situation may obtain because of the comparatively large number of passages these strains have undergone in adult mice. Nevertheless, the experience in this laboratory has been that with these 3 strains the results in 2–6 day old mice are more sharply delineated than are those obtained in adult mice.

Studies on the New Guinea strains, in contrast to those on the 3 strains just discussed, have progressed slowly up to the present because of the low virulence of these strains for adult mice. However, as brought out in this report, only a small number of passages was required to adapt the New Guinea strains to infant mice to the extent that work with these strains could be carried out as effectively as with other strains previously adapted to adult mice.[4]

The neutralization tests in 3–5 day old mice were highly satisfactory from the technical standpoint, and gave results which could be duplicated with considerable regularity. On the basis of cross-neutralization tests, it was found that the 4 virus strains examined by this method fall into one or another of 2 immunologic groups. These findings support those of Sabin (7), who found, by means of dermal neutralization tests and cross-immunity tests in man, neutralization tests in mice, and by complement-fixation tests with convalescent human and monkey sera, that there exist at least 2 immunologic types of dengue virus.

SUMMARY

1. The New Guinea C and D strains were readily adapted to unweaned mice after a relatively small number of serial intracerebral passages.

2. Work with dengue virus may be greatly facilitated by using unweaned mice rather than older animals for isolation and passage, and, with certain strains, for neutralization tests.

[4] Since this paragraph was written, we have learned from Dr. Sabin (8) that the 18th passage of our "baby mouse strain" of New Guinea C virus has yielded titers of $10^{-7.8}$ in 4 week old mice. This is also evident in Table 4, where it will be observed that the infectivity titer of the virus after incubation at 37°C for 2 hours was quite high ($ID_{50} = 1 \times 10^{-6.8}$) in the 4 week old control mice, indicating that the virus is well adapted to propagation in adult animals. Similarly, Dr. R. W. Schlesinger, after successfully establishing the New Guinea B strain in unweaned mice, has been able to adapt this "baby mouse strain" to growth in the mature mouse (9).

3. The 5 strains of dengue virus studied behaved relatively uniformly in unweaned mice 2–6 days of age but differed sharply in their behavior in mice 3–4 weeks old.

4. Cross-neutralization tests in mice corroborated earlier observations that the New Guinea C and D strains are immunologically different from the Hawaiian and Mochizuki strains.

REFERENCES

In future issues of this Journal, references will be cited by date and not by number.

1. KIMURA, R., AND HOTTA, S., Studies on dengue, *Nippon Igaku Oyobi*, Kenko-Hoken, No. 3344:1378, 1943. (Cited from Sabin, reference 7.)
2. SABIN, A. B., AND SCHLESINGER, R. W., Production of immunity to dengue with virus modified by propagation in mice, *Science* **101**: 640–642, 1945.
3. SABIN, A. B., Dengue. Chapter in Viral and Rickettsial Infections of Man, T. M. Rivers, Ed. J. B. Lippincott Co., Philadelphia, pp. 445–453, 1948.
4. SABIN, A. B. 1950 Personal communication.
5. MEIKLEJOHN, G., SIMPSON, T. W., AND STACY, I. B., Dengue antibody in South Pacific residents, *Proceedings of the 7th Pacific Science Congress*, Christchurch, New Zealand, Feb. 1949, in press.
6. MEIKLEJOHN, G., AND SHON, C. W., 1948 Unpublished data.
7. SABIN, A. B., The dengue group of viruses and its family relationships, *Bact. Rev.*, **14**: 225–232, 1950.
8. SABIN, A. B., 1951 Personal communication.
9. SCHLESINGER, R. W., AND FRANKEL, J. W., Adaptation of the New Guinea B strain of dengue virus to suckling and to adult Swiss mice. A study in viral variation, *Am. J. Trop. Med. & Hyg.* **1**: 66–77, 1952.

ADAPTATION OF DENGUE VIRUS TO THE HAMSTER

GORDON MEIKLEJOHN, BEATRICE ENGLAND, AND EDWIN H. LENNETTE

Viral and Rickettsial Disease Laboratory, California State Department of Public Health[1]

The initial report of Sabin and Schlesinger (1) describing the isolation of dengue virus in mice, and subsequent reports (2, 3) describing the properties of the virus, mention that this agent was not found pathogenic for hamsters. Adult hamsters were used, and the lack of pathogenicity of the virus for this species was regarded as a criterion for differentiating dengue virus from other agents, such as Theiler's mouse encephalomyelitis virus and the rodent-adapted strains of poliomyelitis virus. It is for this reason of interest to report that through the use of very young animals, it has proved possible to adapt 2 strains of dengue virus, namely the New Guinea C and Hawaiian strains, to the hamster.

MATERIALS AND METHODS

Material used for hamster passages.—The New Guinea C strain was supplied by Dr. Albert Sabin in the form of lyophilized serum from a patient with dengue. As reported elsewhere (4), this strain was adapted to mice in this laboratory by serial intracerebral passage in mice 2 to 4 days of age. Mouse brains of the tenth passage level were used to initiate the hamster passage line.

In the case of the Hawaiian strain, one hundred and fourth mouse brain passage virus was used to start the hamster passage line.

Procedure for passaging virus.—For passage purposes, infected brains removed from moribund animals were ground in a mortar without abrasive and made to a 20 per cent suspension by gradual addition of 0.85 per cent saline containing 30 per cent of normal rabbit serum (inactivated at 60°C for 30 minutes). This suspension was centrifuged for 30 minutes at 3,000 r.p.m. in the angle head of a refrigerated centrifuge. The supernatant fluid was then drawn off and used as the inoculum. Unweaned hamsters (ages given in the several tables) were inoculated by the intracerebral route and except for very small animals which received 0.02 ml., the usual volume of inoculum employed was 0.03 ml.

Syrian hamsters (*Cricetus auratus*) of known age were supplied by a local breeder with whom special arrangements had been made for this purpose. When animals of suitable age were not available, and passage of the viruses had to be interrupted, passage material, either in the form of whole brain or brain suspension, was stored in flame-sealed ampoules on dry ice.

Neutralization tests.—All sera used were inactivated at 56°C for 20 minutes prior to use; although the addition of fresh, unheated normal serum to inactivated sera has been recommended by Sabin (2) to replace an "accessory factor" destroyed by heating, this was not done in the tests reported here. The neutralization technic employed was that described elsewhere (4). Briefly, 20 per cent suspensions of infective brain material, prepared as described above, were used

[1] 1392 University Avenue, Berkeley 2, California.

to prepare a series of 10-fold dilutions in 30 per cent normal rabbit serum-saline. To an aliquot of each virus dilution was added an equal volume of the serum under test. Specific immune and known negative sera were included as controls. The serum-virus mixtures were incubated at 4°C for 4 hours and then inoculated intracerebrally into unweaned hamsters or mice in 0.02 ml. amounts.

Complement-fixation test.—The technic employed was that used routinely in this laboratory and has been described elsewhere (5). Antigens for the complement-fixation tests were prepared both from mouse brain and hamster brain material. The brains were ground in a mortar and made to a 20 per cent suspension by gradual addition of 0.85 per cent saline. The suspension was kept overnight in a refrigerator at 4°C and then centrifuged horizontally at 2,000 r.p.m. for 30 minutes. The supernatant fluid was drawn off, alternately frozen and thawed 6 to 8 times in an alcohol-dry ice bath, and then centrifuged at 10,000 r.p.m. for 1 hour in a refrigerated, high speed preparative-type centrifuge. The resultant supernatant fluid, after addition of merthiolate to a final concentration of 1:10,000, was used as the antigen.

Appropriate controls, including a 4 tube complement control, were included in the complement-fixation tests.

RESULTS

New Guinea strain.—In the first passage, 1 litter of hamsters, comprising 3 animals 3 days of age, was inoculated. Six days after inoculation 1 animal was found dead. The remaining 2 animals were ataxic and showed a coarse tremor; they were killed and the brains used for passage. The virus was carried through an additional 7 passages, as indicated in table 1.

Animals 4 or less days of age usually began to appear ill and show signs of central nervous system infection by the fifth or sixth day after inoculation, and died by the eighth or ninth day. A summary of the results of the first 8 passages is presented in table 1.

At the third, fifth, and eighth passages, older hamsters were inoculated with the same passage-virus suspension in order to ascertain whether adaptation to unweaned animals had conferred on the virus the ability to induce lethal infections in mature animals. Animals of approximately 3 weeks of age, and of 8 weeks of age were used, the younger animals being used at the third and fifth passages, the older animals at the eighth passage. The results are presented in table 2, which shows that the virus was not pathogenic for animals 17 days of age or over. Since only virus of low passage level in unweaned animals was used, however, these findings do not preclude the possibility that with continued passage in suckling hamsters, the virus may acquire lethality for the adult animal.

To verify that the agent being passaged was dengue virus and not a latent agent of the host, a small-scale, orientative neutralization test was done in 3 day old hamsters, using second hamster passage material. As is shown in table 3, the agent was neutralized by serum from a monkey inoculated with New Guinea C mouse brain virus (4), indicating that the agent being passaged actually was

dengue virus. Table 3 is also of interest in that it shows that neutralization tests with dengue virus can be satisfactorily conducted in baby hamsters.

As a further check that dengue virus was being passaged, suspensions of hamster brains from the sixth and eighth passages were tested against dengue virus antisera in neutralization tests using 4–5 day old mice. The LD_{50} titers of the brain suspensions at the 2 passage levels were $10^{-6.3}$ and $10^{-6.5}$ respectively. The

TABLE 1

Results of serial intracerebral passage of the New Guinea C strain of dengue virus in hamsters

PASSAGE NUMBER	AGE OF ANIMALS	DAY ON WHICH ANIMAL DIED OR WAS KILLED*	MORTALITY RATIO†
	days		
1	3	D6, K6, K6	3/3
2	3	K6, K6, K6, K6, K6, K6, K6	7/7
3	3	K6, K6, D8, D8, D8, D9	6/6
4	10	K8, K8, K8, D10, S, S, S, S	4/8
5	4	K6, K7, K7, K7	4/4
6	2	K5, K5, K5, K5, K5, K5, K5, K5	8/8
7	3	K4, K4, K4, K7, K7, K7, D7, D7, D8, D8, D8, D9, D9	13/13
8	5	D7, D8, D8, D9, D9, D9, D9, D9, K9, K9	10/10

* D—Died.
K—Killed when moribund.
S—Survived without evidence of illness during observation period of 28 days.
† Numerator indicates number of animals which died (or were killed when moribund); denominator indicates number of animals inoculated.

TABLE 2

Comparison of lethality of New Guinea C hamster passage dengue virus in animals of different ages

VIRUS AT PASSAGE LEVEL	UNWEANED HAMSTERS		OLDER HAMSTERS	
	Age	Mortality ratios	Age	Mortality ratios
	days			
3	3	6/6	20 days	1/8
5	4	4/4*	17 days	0/10
8	5	10/10	8 weeks	0/8
8	8	8/8		

* Animals killed when moribund.

results of the tests are shown in table 4. It will be seen that the virus was neutralized by the sera of monkeys immunized with human or mouse-adapted strains of the New Guinea virus, but not by human or monkey sera containing antibodies to the Hawaiian strain. These results show not only that dengue virus was being propagated in the hamster, but also that the hamster passage line of the New Guinea strain is identical with the mouse passage line of this strain, which is also neutralized by New Guinea C strain antisera (4). Finally, the results with heterologous sera, shown in table 4, support the previous observa-

tions recorded by Sabin (2) and by us (4) that the New Guinea C strain is immunologically different from the Hawaiian strain.

Hawaiian strain.—A suspension of infected brains from the one hundred and fourth mouse passage was used for the first hamster passage. Adaptation to the hamster was accomplished less readily than with the New Guinea strain, but

TABLE 3

Verification that agent in second hamster passage brain material was dengue virus. Neutralization tests in 3 day old hamsters

DILUTION OF INFECTED BRAIN	MONKEY SERUM	RESULTS IN TEST HAMSTERS	
		Day of death	Mortality ratio
10^{-1}	Normal	7, 7, 8, 8, 8, 9	6/6
10^{-2}		7, 7, 8, 8, 9, 11	6/6
10^{-1}	New Guinea C immune	12, 15, 18, 19, S*, S, S	4/7
10^{-2}	(Monkey 012)	15, S, S, S, S, S	1/6

* Survived.

TABLE 4

Results of neutralization tests in 4–5 day old mice, using hamster-adapted strains of dengue virus

ANTISERUM		NEUTRALIZATION INDEX WITH	
Strain	Source	New Guinea C strain dengue virus*	Hawaiian strain dengue virus†
New Guinea C	Monkey 012[1]	425	0
New Guinea C	Monkey 934[2]	347	7
Hawaiian	Human (#3507)[3]	0	1514 or >
Hawaiian	Human (E. C.)[4]	6	295
Hawaiian	Monkey 013[5]	5	525

* LD_{50} in 4–5 day old mice = $10^{-6.2}$ to $10^{-6.5}$.

† LD_{50} in 4–5 day old mice = $10^{-4.6}$ to $10^{-4.9}$.

[1] Monkey was immunized with mouse-adapted New Guinea C strain of virus (4).

[2] Immunized with acute phase serum from patient with dengue; this monkey immune serum was supplied by Dr. A. B. Sabin.

[3] Serum from patient in seventh week of convalescence; supplied by Dr. W. McD. Hammon.

[4] Serum from individual 3 months after attack of dengue; supplied by Dr. Sabin.

[5] Monkey immunized with mouse-adapted Hawaiian strain of virus (4).

by the fifth passage the animals succumbed uniformly, and generally by the twelfth day (table 5). It will be noted in table 5 that at the second and seventh passages, animals of 9 days and 20 days of age were inoculated in parallel with the young animals (2 and 3 days of age) employed for the passage series. It is of interest that the older animals were resistant, and that resistance to the lethal effects of the virus apparently appears at least as early as the ninth day of age.

Titration of the brains from the fourth and eighth passage levels gave intracerebral LD_{50} endpoints of $10^{-4.9}$ and $10^{-4.6}$ respectively. These same suspensions were used in neutralization tests in 4 to 5 day old mice to check on the identity of the virus being passaged. As shown in table 4, the virus was neutralized by Hawaiian strain antisera but not, as would be expected in view of what has been said above, by the New Guinea C strain antisera.

TABLE 5

Results of serial intracerebral passage of the Hawaiian strain of dengue virus in hamsters

PASSAGE NUMBER	AGE OF ANIMALS	DAY ON WHICH ANIMAL DIED OR WAS KILLED*	MORTALITY RATIO
	days		
1	3	K6, K7, K7, D11, S, S	4/6
2	3	K7, K12, K12, S, S, S	3/6
2	20	K7, S, S, S, S, S, S, S, S	1/9
3	4	K8, K8, S, S, S, S	2/6
4	3	K7, K10, K10, D13, D13, D18, D18, S, S, S	7/10
5	2	K7, K7, D10, D11, D11, D12, D12, D12, D12	9/9
6	3	K9, D12, D15	3/3
7	2	D6, D9, D10, D12, D12, D12, S	6/7
7	9	S, S, S, S, S, S, S	0/7

* D—Died.
K—Killed when moribund.
S—Survived during observation period of 28 days.

TABLE 6

Identification of hamster-adapted dengue virus by means of the complement-fixation test

ANTIGEN	IMMUNE SERUM FROM	TITER
Hamster brain (New Guinea C strain)	Monkey inoculated with mouse-adapted New Guinea C strain.	1:512
Mouse brain (New Guinea C strain)	Hamsters inoculated with hamster-adapted New Guinea C strain.	1:128
Hamster brain (Hawaiian strain)	Monkey inoculated with mouse-adapted Hawaiian strain.	1:128
Mouse brain (Hawaiian strain)	Hamsters inoculated with hamster-adapted Hawaiian strain.	1:256

Identification of hamster passage strains by means of the complement-fixation test.—Further identification of the agents under passage as being dengue virus was supplied by the results of complement-fixation tests, in which antigens prepared from hamster brains were tested against immune sera produced in monkeys by inoculation with the homologous mouse-adapted strain, and antigens prepared from mouse passage lines of the virus were used to test antisera produced in hamsters inoculated with the hamster passage virus. The results are summarized in table 6. It will be seen that antigens prepared from the hamster-adapted lines of the New Guinea C and Hawaiian strains fixed complement in the presence of homologous immune sera from monkeys immunized with New Guinea C and

Hawaiian mouse passage viruses respectively. Similarly, immune sera prepared by inoculation of hamsters with hamster passage New Guinea C or Hawaiian virus gave fixation when tested with homologous antigens prepared from mouse brains representing the mouse passage line of these strains.

COMMENT

Attempts to adapt the dengue virus to suckling hamsters were prompted by two factors: (a) the observation that very young unweaned mice are considerably more susceptible to dengue virus than are older animals (4, 6) and (b), experience with a number of viruses in this laboratory in which primary isolation, or adaptation, has been accomplished more readily in the younger animals. These viruses include strains of St. Louis encephalitis virus (7, 8), western equine encephalomyelitis virus (9), influenza B (10), herpes simplex (11), and Newcastle disease virus (12). Casals and Olitsky (13) have recently reported similar observations with still other viruses.

It would appear that dengue virus should no longer be described as nonpathogenic for hamsters. While it is true that adult animals are relatively insusceptible, the New Guinea C and Hawaiian strains, representing each of the 2 main types, are lethal for very young animals and multiply in the brain to a degree not greatly inferior to that observed in mice of similar age. The factor of age as well as animal species is of importance and deserves consideration, as has been emphasized by Lennette (7), in defining the host range of a given virus.

SUMMARY

By serial intracerebral passages, the New Guinea C and Hawaiian strains of dengue virus were adapted to growth in hamsters of 2–5 days of age.

Dengue virus should no longer be described, without qualification, as nonpathogenic for the hamster.

Additional data are presented showing that the New Guinea C and Hawaiian strains represent 2 different immunologic types of dengue virus.

REFERENCES

In future issues of this Journal, references will be cited by date and not by number.

1. SABIN, A. B., AND SCHLESINGER, R. W., Production of immunity to dengue with virus modified by propagation in mice, *Science* **101**: 640–642, 1945.
2. SABIN, A. B., The dengue group of viruses and its family relationships, *Bact. Rev.* **14**: 225–232, 1950.
3. SCHLESINGER, R. W., Propagation in chick embryos of the Hawaiian strain of dengue virus. I. Sustained serial passage in eggs after 101 intracerebral passages in mice, *Am. J. Hyg.* **51**: 248–254, 1950.
4. MEIJKLEJOHN, G., ENGLAND, B., AND LENNETTE, E. H., Propagation of dengue virus strains in unweaned mice, *Am. J. Trop. Med. & Hyg.* **1**: 51–58, 1952.
5. LENNETTE, E. H., CLARK, W. H., AND DEAN, B. H., Sheep and goats in the epidemiology of Q fever in Northern California, *Am. J. Trop. Med.* **29**: 527–541, 1949.
6. MEIKLEJOHN, G., SIMPSON, T. W., AND STACY, I. B., Dengue antibody in South Pacific

residents. Proceedings of the 7th Pacific Science Congress, Christchurch, New Zealand, Feb. 1949, in press.

7. LENNETTE, E. H., Isolation of St. Louis encephalitis virus from a fatal human case in California, *Proc. Soc. Exptl. Biol. and Med.* **61**: 206–210, 1946.
8. LENNETTE, E. H., unpublished observations.
9. MEIKJLEJOHN, G., ENRIGHT, J. B., AND LENNETTE, E. H., unpublished observations.
10. MEIKLEJOHN, G., ENGLAND, B., AND LENNETTE, E. H., unpublished observations.
11. LENNETTE, E. H., AND BRUNETTI, O., unpublished observations.
12. MEIKLEJOHN, G., AND KUNSCH, D., unpublished observations.
13. CASALS, J., AND OLITSKY, P. K., A complement fixation test for poliomyelitis virus, *Proc. Soc. Exptl. Biol. and Med.* **75**: 315–318, 1950.

ADAPTATION OF THE "NEW GUINEA B" STRAIN OF DENGUE VIRUS TO SUCKLING AND TO ADULT SWISS MICE

A Study in Viral Variation.[1]

R. WALTER SCHLESINGER and JACK W. FRANKEL

Division of Infectious Diseases, The Public Health Research Institute of The City of New York, Inc., New York 9, N. Y.

Adaptation of the Hawaiian strain of dengue virus to growth in the brain of young adult Swiss mice was a gradual process (1). When serial transfers were made with 10 per cent mouse brain suspensions serving as inocula, it required 15 passages before all of the mice became paralyzed. Once paralysis and death occurred regularly, the titer of the virus could be calculated, and after many further passages it increased from an initial value of about 10^2 to about 10^5 LD_{50} per 0.03 gram of brain tissue. Subsequently, following a suggestion by Dr. Gordon Meiklejohn, the mouse-adapted Hawaiian strain was propagated in 3 to 4 day old mice, and this yielded 100–1,000 times higher infectious titers. Furthermore, infected baby mouse brain was found to be a good source of complement-fixing antigen (2).

From 1944 to 1946, Sabin and Schlesinger attempted in a number of ways to adapt to mice 3 other strains of dengue virus, i.e. the New Guinea "B", "C" and "D" strains which were recognized as being immunologically distinct from the Hawaiian type (see (3)). Partial success was obtained when the viruses were inoculated intracerebrally into mice of the dba strain. It was also noted that primary "takes", i.e. evidence of infection in the form of weakness or paralysis of the extremities, were more apt to follow inoculation of dilute than of undiluted or concentrated virus from human sources (1).

Stimulated by the usefulness of the suckling mouse in enhancing the infectivity of the Hawaiian strain, we decided to attempt reactivation of a series in which the New Guinea "B" strain had been carried through eight successful passages in dba mice. It has been possible to adapt this strain to Swiss baby mice. This process of adaptation has been closely analogous to that reported by Meiklejohn *et al.* (4) for the New Guinea "C" and "D" strains. Since these investigators were unable to obtain similar results with the "B" strain, it is one of the purposes of this communication to round out the present status of known types of dengue virus in relation to pathogenicity for mice and to serological group relationship. The second purpose is to describe the gradual acquisition by the virus of pathogenicity for adult Swiss mice as well, a process which by its nature raises several points of general interest in relation to the problem of viral variation in the course of adaptation to new hosts.

[1] This investigation was supported by a research grant from the National Institutes of Health, U. S. Public Health Service.

MATERIALS AND METHODS

Virus strains: The Hawaiian and the New Guinea "B", "C" and "D" strains of dengue virus were available in the form of sera obtained from experimentally infected volunteer patients during the first 24 hours after onset of fever. Such sera were used for primary inoculation into mice and for immunization of monkeys as described below.

The mouse-adapted strain of Hawaiian dengue virus was used in the form of infected baby mouse brain. Mice were inoculated on the first to fourth day of life. They were sacrificed when showing paralysis or other signs of encephalomyelitis. Stock virus consisted of 20 per cent suspensions of infected brain in 50 per cent heated (56°C. for 30 minutes), Seitz-filtered, normal rabbit serum in saline which were stored in the solid CO_2 box. In most experiments, however, freshly prepared virus was used, because freezing usually resulted in a slight loss of infectivity.

Virus titrations: Tenfold serial dilutions of virus were prepared in saline containing 10 per cent heated normal rabbit serum. Of these, 0.03 ml. were inoculated intracerebrally into adult mice and 0.01 or 0.02 ml. into suckling mice. Where possible titration endpoints were calculated and will be expressed in terms of LD_{50} per 0.01 to 0.03 gm. of brain tissue, depending on the volume inoculated.

Preparation of immune sera: Potent immune sera were obtained from rhesus monkeys which had received a single intracerebral inoculation of 1.0 ml. of acute phase human serum as suggested by Sabin and Young (2). Through the courtesy of Dr. F. B. Bang, Johns Hopkins Hospital, Baltimore, Md., convalescent serum was also obtained from a volunteer patient (W. H.) who had been experimentally infected with human New Guinea "B" virus.

Neutralization tests: All neutralization tests reported in this paper were carried out as follows: Sera to be tested were heated at 56°C. for 30 minutes. To 0.15 ml. amounts of 1:4 diluted, heated serum, 0.15 ml. of native guinea pig serum was added. This treatment was used in order to minimize the non-specific virus inactivating effects to which Sabin (5) has called attention. These mixed sera were added to 0.3 ml. of serial virus dilutions (tenfold decrements of 2×10^{-1}). The virus-serum mixtures were incubated for 2 hours in a 37°C. water bath prior to intracerebral inoculation.

Complement-fixation tests: Antigens were prepared from infected baby mouse brain according to the acetone-ether extraction method of Casals (6). All test sera were diluted 1:4 prior to inactivation at 60°C. for 20 minutes. Tests were set up with 0.1 ml. of antigen, 0.1 ml. of serum dilution and 0.2 ml. of diluted guinea pig serum containing 2 hemolytic units of complement. The tubes were incubated overnight at 4°C. and then 0.2 ml. of 3 per cent sensitized sheep erythrocytes was added. The tests were read after 30 minutes of incubation in the 37°C. water bath. Titers will be expressed in terms of the highest dilution of serum resulting in complete or partial (2 plus) fixation.

Mice: All Swiss mice used in this laboratory were supplied by Tumblebrook Farms, Brant Lake, New York. "Adult" mice were approximately 3 to 4 weeks

old and weighed 8 to 10 grams when used. Pregnant mice of the same stock were purchased from the dealer, and their litters were used 1 to 7 days, usually 2 days, after birth.

EXPERIMENTAL

A. Initial Passages of New Guinea "B" Virus in dba Mice

The work reported in this section was carried out in 1945–1946 by one of us (R. W. S.) in association with Dr. Albert B. Sabin at the Children's Hospital Research Foundation, Cincinnati, Ohio.[2] We are indebted to the latter for permission to summarize the results in this paper. Five attempts to propagate the New Guinea "B" strain by passage in 2 to 3 week old Swiss mice had been unsuccessful. In a further trial, 0.02 ml. of acute phase human serum was inoculated intracerebrally into each of eight dba mice.[3] Three of these showed weak extremities 22, 24 and 29 days, respectively, after inoculation and were sacrificed. Because dba mice were not available, three parallel sub-passages were done in 3 week old Swiss mice. One out of a total of 28 mice died after 6 days without previous signs of illness. Of the remaining 27 mice, three showed questionable or transitory weakness of the extremities beginning on the 9th to 22nd day after inoculation. Further passages in Swiss mice were negative. Three months later, dba mice were again available, and at this time the brain suspension derived from one of the Swiss mice of the second passage, which had been stored in the dry ice box, was thawed out and used for further passage. This time, 3 of 8 dba mice showed definite signs: one died and two were sacrificed for passage. In the fourth passage, all but 4 of 18 dba mice showed definite paralysis or weakness, and of these six were sacrificed and two died. Four additional serial passages were carried out in dba mice, but there was no evidence of an increase in pathogenicity up to the eighth passage. Altogether, approximately 50 per cent of the mice displayed weakness or paralysis of the limbs. Occasional transfers were done simultaneously in dba and in 3 week old Swiss mice. Two of a total of 28 Swiss mice died without having exhibited characteristic signs. All others remained well.

In March, 1946, work on this problem was temporarily discontinued. Materials derived from dba mice in the fourth and eighth passages were dried from the frozen state. A few months later, an attempt was made by Dr. Sabin to reactivate the series by inoculating re-constituted brain suspension of the eighth passage into dba mice, but the result was negative.

B. Intracerebral Passages in Baby Swiss Mice

In February, 1951, a 20 per cent suspension of brain tissue from the fourth dba mouse passage, which had been lyophilized 5 years previously, was reconstituted and inoculated into eight 3 day old Swiss mice. Two of these showed

[2] This part of the work was done under the auspices of the Commission on Neurotropic Virus Diseases, Army Epidemiological Board, U. S. Army.

[3] Mice of the dba strain (dilute brown, non-agouti) were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

questionable signs of weakness eight days later and were sacrificed for passage. The other animals remained well for 28 days. A 10 per cent suspension of the brains of the sacrificed mice served as inoculum in the next passage, designated as M6. All of the 9 mice in this passage were paralyzed after 7 to 9 days. Further serial passages with 10^{-1} or 10^{-4} diluted mouse brain invariably produced paralysis and death. The lethal titer of material from passage M9 was at least $10^{-6.5}$. Titrations carried out after further passages in 1 to 7 day old mice yielded endpoints of the order of 10^{-7} to $10^{-8.6}$ (Table 1). The illness caused in baby mice was characterized by partial or complete paralysis of the extremities, often preceded by marked spasticity or ataxia and accompanied by convulsive seizures. Affected mice usually died within 24 hours after onset. Death occurred, as a rule, 6 to 9 days after inoculation depending on the amount of virus inoculated.

TABLE 1

Titrations in baby mice

INOCULUM: BABY MOUSE PASSAGE NUMBER:	MORTALITY RATE AMONG MICE INOCULATED WITH DILUTIONS:									LOG LD_{50}
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	
M9	—	4/4	6/6	11/11	5/5	6/6	—	—	—	>6.5
M12	6/6	7/7	8/8	8/8	7/7	6/6	7/7	7/7	—	>8.5
M17	—	—	—	—	5/5*	2/2*	4/8	2/9	0/4	7.2
M20	3/3	6/6	†	7/7	7/7	8/8	2/4	4/4	2/4	8.6
M22	4/4	5/5	4/4*	6/6	6/6	6/6	8/8	8/8	1/5	8.6
M25	7/7	7/7	7/7	6/6*	6/6	7/7	8/9	0/2	—	7.9

* Additional mice with CNS signs sacrificed for passage.

† All mice sacrificed when showing CNS signs.

C. Progressive Adaptation to Adult Swiss Mice

(1) by serial passage through suckling mice: At different passage levels, the baby mouse-adapted virus was titrated in 3 to 4 week old Swiss mice of the same breed (Tumblebrook Farms). Initially, i.e. after a total of 6, 9 or 12 passages in mice, the incidence of signs in older mice was low and irregular as shown in Table 2. After 15 passages, the mortality rate was increased but remained apparently independent of the amount inoculated. From the 17th through the 24th baby passage, the pathogenicity of the virus for adult mice changed gradually, especially in that fewer mice survived once they had become paralyzed. The lack of stability of the factor responsible for pathogenicity in adult mice is evident from the titration of the sample from passage M21 (Table 2) which gave very erratic results.

A distinct change was noted after a total of 25 passages. As seen in Table 2, it was from this stage on that baby mouse brains were consistently infectious and lethal for adult Swiss mice, and that endpoints could be calculated. The titers were closely analogous in suckling and in adult mice. The illness pro-

duced in adult mice consisted of flaccid paralysis of the extremities, beginning usually 6 to 12 days after inoculation, with death occurring 1 to 2 days later.

(*2*) *by serial passages in adult mice:* It was thought that adaptation to adult Swiss mice might be facilitated by serial intracerebral transfers in such animals. The first attempt in this direction was made in a series initiated by inoculating varying amounts of baby mouse virus from the 6th passage into adult mice. Definite paralysis occurred in 6 of 15 mice inoculated with the three highest dilutions (i.e. 10^{-5} to 10^{-7}), although dilutions 10^{-2} to 10^{-4} were innocuous.

TABLE 2

Titrations of infected baby mouse brain at different passage levels by intracerebral inoculation in adult mice

VIRUS SAMPLE		MORTALITY (AND MORBIDITY)* RATIO AMONG MICE INOCULATED WITH DILUTIONS:									LOG LD_{50} IN ADULT MICE
Baby mouse passage no.	Log LD_{50} in baby mice	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	
M6	—	2/4	0/5	1/5(?)	0/4	1(2)/5	1(2)/5	0(2)/5	0/5		×
M9	>6.5	1/4	2/5	1/5	0/4	1/4	1(2)/4				×
M12	>8.5	2/5	0/5	2(3)/5	1/5	1/4	2(3)/5	1/5	0/5		×
M15	—	4(6)/7	4(6)/8	2/8	4(6)/8	7/8	7/8	2/8			×
M17	7.2	3(4)/5	3/5	5/5	2(5)/5	4/4	3(5)/5	2(3)/5	0/5		×
M18	7.0	2/2	4/5	4/4	4(5)/5	5/5	4/4	1(2)/5	0/5	0/4	×
M20	8.6	2/3	4/5	2(3)/5	3(4)/5	5/5	5/5	2/5	0/5	0/4	×
M21	6.9		1/5	2/5	0(1)/5	2/5	4/5	2/5	0/5		×
M22	8.6		5/5	4(5)/5	4/5	3/5	3/4	4/4			×
M23	7.9	5/5	4/5	5/5	5/5	2(3)/5	5/5	1(2)/5	0/5	0/5	×
M24	7.4	5/5	5/5	2(3)/3	5/5	3(4)/5	1(2)/5	2/5	0/5		×
M25	7.9	3/3	4/4	4/4	4/4	4/5	4/5	3/5	0/5	0/5	6.7
M26	8.2	4/4	4/4	4/4	4/4	4/4	4/4	4/4	1/4		7.7
M27	8.0	5/5	5/5	5/5	5/5	5/5	5/5	4/5	1/5		7.5
M28	7.1	8/8	8/8	8/8	8/8	8/8	7/7	4/8	0/7		7.0
M29	>7.5	5/5	5/5	5/5	4/4	5/5	5/5	5/5	0/5		7.5
M35	7.8	5/5	5/5	5/5	5/5	5/5	5/5	4/4	0/5		7.5

* Figures in parentheses indicate, in addition to those mice which died, those which were sacrificed when showing CNS signs or which survived for 21 days after showing typical CNS signs for several days. Numbers of mice observed are indicated in denominator.

× LD_{50} not calculated because of irregular results.

In subsequent adult-to-adult transfers, brains of mice inoculated with relatively small doses of virus were preferably harvested and titrated in turn. It may be seen in Table 3 that 12 serial passages failed to lead to increased virulence: the outcome of titrations remained erratic, although the cumulative incidence of definite signs was highest among those mice which received 10^{-4} diluted mouse brain (41 per cent). The possible significance of the latter point will be discussed below.

Two additional series were initiated in adult mice by inoculating baby mouse brain derived from the 18th and 20th passages, respectively. In both series,

adult-to-adult transfers were done, as a rule, with brain tissue at a dilution of 10^{-2}. Here, as in the progressive adaptation to adult mice by passage in babies, the transition of the virus to a form which was invariably fatal for adult mice and yielded clear-cut titration endpoints occurred after a total of 26 to 27 passages in dba, baby and adult Swiss mice. Even at this stage, the virus titer of infected adult mouse brain was consistently lower than that of baby mouse brains, regardless of whether the titrations were done in adult or baby mice. One such comparative titration is presented at the bottom of Table 4. It is seen that virus derived from adult mice after a total of 27 passages had a lethal

TABLE 3

Adult-to-adult-mouse passages

ADULT PASSAGE NO.	INOCULUM	TOTAL NO. OF PASSAGES	COMBINED INCIDENCE OF DEATH AND CNS SIGNS AMONG MICE INOCULATED WITH DILUTIONS:							
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Ad. 1	M6 (baby)	7	2/4	0/5	1?/5	0/4	2/5*	2/5	2/5	0/5
Ad. 2	Ad. 1	8	0/5	0/5	4/5*	4/5	0/5	—	—	—
Ad. 3	Ad. 2	9	0/5	0/5	2/5	2/5	2/4*	—	—	—
Ad. 4	Ad. 3	10	1/4*	1/5	0/5	2/5*	2/5	—	—	—
Ad. 5	Ad. 4	11	0/4	1/5	2/5*	4/5*	1/5	0/5	—	—
Ad. 6	Ad. 5	12	2/5	0/5	3/5*	3/5*	2/5	0/5	—	—
Ad. 7	Ad. 6	13	3/3*	4/5*	2/5	1/4	1/5	0/5	0/5	0/5
Ad. 8	Ad. 7	14	1/4	1/5	2/5*	1/5	2/5	1/4	0/5	0/4
Ad. 9	Ad. 8	15	3/5*	1/4	0/5	1/5	0/5	0/5	0/5	0/5
Ad. 10	Ad. 9	16	1/4	0/5	0/5	2/5*	2/5	0/5	0/5	0/5
Ad. 11	Ad. 10	17	0/5	0/5	0/5	0/5	0/5	1/4*	0/5	0/4
Ad. 12	Ad. 11	18	—	0/10	—	—	—	—	—	—
Total for Passages Ad. 2–11†			11/44	8/49	15/50	20/49	12/49	2/33	0/25	0/23
Percent			25	16	30	41	25	6	0	0

* Virus harvested from mice in these groups was titrated in each subsequent passage.

† Passage Ad. 1 is not included in the total because the inoculum, being derived from baby mice, was not comparable with those of subsequent passages.

titer of $10^{-5.6}$ in adult mice and 10^{-6} in suckling mice. In this respect, the New Guinea "B" strain was similar to the Hawaiian strain (2).

Of greater interest are, however, the other tests recorded in Table 4 which reveal that virus harvested from earlier passages in adult mice, while highly erratic in its behavior in adult mice, was consistently infective and lethal for baby mice. Thus it is clear that adult mice were capable of supporting the multiplication of a viral type to which they themselves were not highly susceptible.

D. Serological Identification of the Mouse-Adapted Strain

(*1*) *Complement-fixation tests:* Preliminary identification of the virus by complement fixation tests was done after a few passages through baby mice with the

TABLE 4

Serial passages in adult mice: comparative infectivity titrations of infected adult mouse brains in adult and in baby mice

SOURCE OF VIRUS TITRATED			AGE OF MICE USED FOR TITRATION	MORTALITY (AND MORBIDITY)* RATES AFTER INOCULATION OF INDICATED DILUTIONS							LOG LD_{50}
Adult passage series	No. of passages in adult mice	Total no. of passages in mice		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	
A	Ad. 3	9	Adult	0(1)/4	1/5	0/5	1(2)/5	2/5	—	—	×
			Baby	6/6	6/6	8/8	5/5	6/6	—	—	>5.5
	Ad. 6	12	Adult	1(3)/3	3(4)/5	0(3)/5	1/4	0(1)/5	0/5	0/5	×
			Baby	5/5	5/6	3/7	0/7	0/3	0/2	0/9	2.8
B	Ad. 6	26	Adult	4(5)/5	3(5)/5	5/5	2/5	1(2)/5	0/5	0/5	×
			Baby	4/4	7/7	6/6	4/4	2/6	0/7	0/5	4.8
C	Ad. 9	27	Adult	5/5	5/5	5/5	5/5	3/3	1/5	—	5.6
			Baby	3/3	6/6	7/7	6/6	7/7	3/6	—	6.0

* Figures in parentheses indicate, in addition to those mice which died, those which were sacrificed when showing CNS signs or which survived for 21 days although showing typical CNS signs for many days.

× LD_{50} not calculated because of irregular results.

TABLE 5

Summary of complement-fixation tests

SERUM			COMPLEMENT-FIXING TITER* OF SERUM IN PRESENCE OF ANTIGEN		
Donor	Strain of virus used for immunization†	Interval between inoculation and bleeding	Dengue N.G. "B"‡	Dengue Hawaii	Normal mouse brain
Rhesus No. 27	New Guinea "B"	0	<4§	<4	<4
		1 month	32–64*	16	<4
Rhesus No. 29	New Guinea "D"	0	<4	<4	<4
		1 month	32–64*	8	<4
Rhesus No. 28	Hawaii	0	<4	<4	<4
		1 month	<4–8*	16	<4
Patient W. H.	New Guinea "B" (experimental disease)	2 d. before onset	<4	<4	<4
		32 d. after onset	16–32*	8	<4

* Titers indicate highest dilutions of serum giving 2 plus or greater fixation. Multiple figures give results of repeated tests with different antigen preparations of the same strain.

† Immunizing agents were in each instance human acute phase sera.

‡ Four different antigens were used.

§ 1:4 was the lowest dilution of serum tested and failed to give fixation.

results summarized in Table 5. Antigens prepared from baby mouse brains derived from the 8th, 9th and 11th passages reacted with the sera of monkeys

TABLE 6

Summary of neutralization tests

EXP. NO.	SERUM*			VIRUS†			
	Donor	Immunizing dengue strain†	Time interval betw. inoculation & bleeding (Mos.)	Dengue New Guinea B		Dengue Hawaii	
				LD_{50} (log)	Neutr. index	LD_{50} (log)	Neutr. index
I	Normal guinea pig	—	—	7.1	—	7.0	—
	Normal rabbit (10%)	—	—	7.0 (7.1)		7.1 (7.0)	
	Rhesus #27	New Guinea B	0§	6.8	2	7.0	0
			1	4.2	800	>6.4	<4
			3	3.2	8,000	>6.5	<3
			6	2.6	30,000	>6.5	<3
	Rhesus #28	Hawaii	0§	7.4	0	6.5	3
			1	n. t.	n. t.	5.2	64
			3	>6.5	<4	3.7	2,000
			6	5.8	20	3.8	1,600
II	Normal guinea pig	—	—	7.4	—	6.6	—
	Rhesus #29	New Guinea D	0§	7.5	0	6.4	2
			1	6.1	20	5.8	6
			6	4.3	1,300	6.0	4
III	Normal guinea pig	—	—	6.7	—	6.4	—
	Rhesus #569‖	New Guinea B	1	3.8	800	6.0	3
	Rhesus #934‖	New Guinea C	1.3	4.3	250	5.4	10
	Rhesus #936‖	New Guinea C	1.3	4.7	100	5.5	8
	Rhesus #570‖	New Guinea D	1.5	4.2	320	5.7	5
	Rhesus #28	Hawaii	6	6.6	0	3.0	2,500
IV	Normal guinea pig	—	—	6.8	—	6.5	—
	Patient W. H. convalescent	New Guinea B	2	<3.6	>1,600	5.5	10

* All sera were heated at 56°C. for 30 minutes and diluted 1:4 before addition of equal parts of unheated normal guinea pig serum. Hence, the neutralization indices are those of 1:8 diluted sera.

† All immunizations were done by intracerebral inoculation of 1.0 ml of human acute phase sera.

‡ Viruses were used in the form of infected baby mouse brain suspensions.

§ Serum obtained immediately prior to inoculation.

‖ These sera were kindly supplied by Dr. A. B. Sabin, Cincinnati.

n. t. Not tested.

immunized with human New Guinea "B" or "D" virus. Sera of monkeys immunized against the Hawaiian strain gave negative tests or yielded lower titers in presence of New Guinea "B" antigens than of Hawaiian antigen. On the other hand, as has been described by Sabin and Young (2), heterologous immune

sera cross-reacted with Hawaiian antigen, although the titers were 2 to 4-fold lower than in presence of homologous antigens. Similarly, convalescent serum of volunteer patient W. H. had a titer of 1:32 in the presence of homologous New Guinea "B" antigen and of 1:8 with the Hawaiian antigen. These tests tentatively identified the mouse-propagated virus as a strain of dengue virus and indicated its distinctness from the Hawaiian strain.

(*2*) *Neutralization tests:* The results of various neutralization tests are summarized in Table 6. All the tests were carried out in adult mice with virus in the form of infected baby mouse brain. The samples of New Guinea "B" virus used represented the 28th to 33rd passages, those of Hawaiian virus the 109th, to 112th, respectively. The technique of the tests has been described under Methods, and it should be remembered that the neutralization indices given in Table 6 are those of 1:8 diluted sera. It is clear that the New Guinea "B", "C" and "D" immune sera reacted alike, in that they neutralized the New Guinea "B" strain but gave negative or equivocal results when tested with the Hawaiian strain. On the other hand, the sera of monkeys immunized with the latter strain failed to neutralize the New Guinea "B" virus. Another point

TABLE 7

Cumulative incidence of CNS signs among baby mice and adult mice inoculated with different amounts of virus derived from the 6th to 21st baby mouse passages

AGE OF MICE		MORBIDITY RATIO AFTER INOCULATION OF DILUTION:							
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
1–7 days	Ratio	20/20	29/29	32/32	32/32	40/40	37/37	17/30	14/31
	% showing signs	100	100	100	100	100	100	57	45
3–4 weeks	Ratio	19/30	20/43	21/42	22/41	27/40	32/41	14/38	0/30
	% showing signs	63	46	50	55	67	78	37	0

of interest is that neither of the monkeys on whom repeated bleedings were performed (Nos. 27, 28 and 29) produced maximum amounts of antibody during the first month after intracerebral inoculation; in each instance, the neutralization indices against the homologous viral type were higher after 3 or 6 months.

DISCUSSION

The existence of at least two immunological types of dengue virus was established several years ago on the basis of dermal neutralization tests in human beings (5) and of neutralization tests carried out in mice with the mouse-adapted Hawaiian strain (Sabin and Schlesinger, unpublished observations) (3). On the other hand, a certain degree of immunological cross relationship between all known strains of the virus was suggested by the results of re-infection experiments on human volunteers who had recovered from an attack of dengue. It was found that resistance to re-inoculation of homologous strains was complete when the interval between the first and second infecting doses was as long as

18 months (5), while resistance to heterologous strains was present only during the first two months and then faded. Complement-fixation tests also revealed antigenic similarities between the Hawaiian and the heterologous New Guinea strains (2).

The successful adaptation of the New Guinea "B", "C" and "D" strains to mice reported in this paper and in that by Meiklejohn, et al. (4) has made it possible to test the immunological relationship of these strains to one another and to the Hawaiian type in both directions. Neutralization tests have revealed that the three New Guinea strains represent one immunological type and that they are clearly distinct from the Hawaiian strain. Thus, it is definitely confirmed that all known strains of dengue virus fall into two immunological categories: one consists of the three New Guinea strains and the other of the Hawaiian, the New Guinea "A", two strains isolated from patients in Calcutta, India, and strains isolated and adapted to mice by Japanese workers (5). It remains to be seen whether propagation of the New Guinea "B", "C" and "D" strains in mice has led to attenuation of their pathogenicity for man, with retention of their immunizing capacity, comparable with that observed in the case of the mouse-adapted Hawaiian virus (1). Attempts are under way to propagate the mouse-adapted New Guinea "B" strain in chick embryos by applying the procedures which yielded good results in the case of the Hawaiian strain (7, 8).

In the present study, an attempt was made to reveal something about the nature of the change whereby the New Guinea "B" strain, in the course of continued serial intracerebral passages in Swiss mice, acquired pathogenicity not only for suckling but also for adult mice. This transformation was gradual and its completion required the same number of passages regardless of whether they were all carried out in baby mice or whether the last few were done in adults. The latter were apparently no better equipped than the former to give rise selectively to particles of the modified (i.e. adult-pathogenic) kind. Titrations carried out in adult mice on baby mouse brain samples derived from the 6th to the 21st passages (Table 2) produced scattered "takes" over a wide range of virus dilutions, suggestive of variations in the susceptibility of the hosts. However, closer analysis of the findings reveals that the incidence of typical illness was 78 per cent among adult mice inoculated with 10^{-6} diluted mouse brain and 37 per cent in those receiving 10^{-7} diluted material. As shown in Table 7, the cumulative incidence among baby mice inoculated with the same dilutions of comparable samples was 100 and 57 per cent, respectively. This would suggest that in the range of limiting dilutions, the chances of adult mice developing signs of illness were only slightly less than those of baby mice. A similar tendency has already been mentioned above in connection with the adult-to-adult passage series presented in Table 3. These calculations argue against heterogeneity of the adult host population in regard to susceptibility to the nonadapted virus samples. They support the belief that these samples were mixtures of two types of viral particles, i.e. those which were pathogenic for baby mice only and those which were pathogenic for mice of either age.

Upon continued passage in mice of either age, it appears that the latter gradually replaced the former. Further work is now in progress to determine whether the lower incidence of illness among mice inoculated with larger doses of the early passage virus may be due to interference of unmodified with modified particles, or whether two types of particles can be separately characterized by some other means.

SUMMARY

The New Guinea "B" strain of dengue virus has been propagated in mouse brain. Initially, the virus was carried through eight serial intracerebral passages in mice of the dba strain. It produced weakness or paralysis in about 50 per cent of these animals. A sample derived from the fourth passage was transferred to Swiss baby mice after it had been lyophilized for five years. After two passages, the virus was consistently infectious and lethal for 1 to 7 day old baby mice. The lethal titer of infected baby mouse brain was of the order of 10^{-7} to 10^{-8}. Upon continued serial transfer in baby mice, the virus gradually acquired an increasing capacity for producing paralysis and death in adult mice as well. After a total of 26 passages, the transformation of the virus into a form consistently pathogenic for adult Swiss mice was complete. The titer in such animals then was similar to that in baby mice. Adult mice infected with samples derived from earlier baby mouse passages also supported viral multiplication, but the virus arising in them was qualitatively similar to the virus inoculated in that it was likewise of low-grade pathogenicity for adult mice but invariably lethal for baby mice. The nature of the change in the character of the mouse-adapted virus has been discussed. It is believed that the virus arising in early baby mouse passages consists of two types of particles, one pathogenic for baby mice only, the other for mice of either age, and that the latter gradually replace the former upon continued adaptation to the mouse brain.

Neutralization tests carried out with the mouse-adapted New Guinea "B" strain indicate that it is immunologically identical with the New Guinea "C" and "D" strains and distinct from the Hawaiian type. It is concluded that all known strains of dengue virus belong to one of two separate immunological types.

REFERENCES

In future issues of this Journal, references will be cited by date and not by number.

1. Sabin, A. B., and Schlesinger, R. W., Production of immunity to dengue with virus modified by propagation in mice, *Science* **101:** 640, 1945.
2. Sabin, A. B., and Young, I., A complement fixation test for dengue, *Proc. Soc. Exp. Biol. and Med.* **69:** 478, 1948.
3. Sabin, A. B., Dengue. In Viral and Rickettsial Infections of Man, T. M. Rivers, Editor. J. B. Lippincott Co., 1948.
4. Meiklejohn, G., England, Beatrice, and Lennette, E. H., Propagation of dengue virus strains in unweaned mice, *Am. J. Trop. Med. and Hyg.* **1:** —— 1952.
5. Sabin, A. B., The dengue group of viruses and its family relationships, *Bact. Reviews* **14:** 225, 1950.

6. CASALS, J., Acetone-ether extracted antigens for complement fixation with certain neurotrophic viruses, *Proc. Soc. Exp. Biol. and Med.* **70:** 339, 1949.
7. SCHLESINGER, R. W., Propagation in chick embryos of the Hawaiian strain of dengue virus. I. Sustained serial passage in eggs after 101 intracerebral passages in mice, *Am. J. Hyg.* **51:** 248, 1950.
8. SCHLESINGER, R. W., Propagation in chick embryos of dengue virus, Hawaiian strain. II. Findings in infected eggs, *Proc. Soc. Exp. Biol. and Med.* **76:** 817, 1951.

ENDEMIC YELLOW FEVER IN PANAMA AND NEIGHBORING AREAS

HERBERT C. CLARK

Director, Gorgas Memorial Laboratory, Panamá

My service on the Isthmus of Panama began December 9, 1909, as pathologist in the Board of Health Laboratory at Ancon, C. Z. So much publicity had been given this region for many years that it seemed likely that yellow fever and plague would crowd out most of the other causes of death. Imagine my surprise to find that malaria, dysentery, tuberculosis and pneumonia were the high ranking causes of death. No cases of yellow fever developed in the Canal Zone or in the terminal cities during my service from 1909 to 1922. However, rare cases arrived at the quarantine stations and sometimes these were transferred to Ancon Hospital (now Gorgas Hospital) for better hospital care and observation. Most of those cases came from the west coast of South America. It is only in fairly recent years that laboratory methods have been developed that help in establishing a diagnosis of yellow fever. There is no doubt that many ambulatory cases escaped the records. The mouse protection test has been a great advance in identifying yellow fever and the viscerotomy service has made it possible to forward liver specimens from fatal cases that occur in remote places where no doctor and no pathologist is in attendance.

From 1929 to 1931 the Gorgas Memorial Laboratory collected blood specimens from a number of adult foreign whites and others who lived and worked in the Darien province of Panama. These specimens were forwarded to the Rockefeller Foundation for protection tests. None of the adult foreign whites were positive but one 9 year old and one 16 year old native child (negroid race) gave positive tests and three others gave doubtful tests.

During the years 1935–1936 Dr. Carl M. Johnson of our staff was sent to Darien to cover several settlements and some Indian locations. The idea was to draw enough blood for a Wassermann test and a laboratory survey of Chagas disease and yellow fever. Johnson sent about 270 human blood specimens to the Rockefeller laboratory in New York, and seven of these gave a positive protection test including two children under 13 years of age. Dr. Johnson then repeated these surveys on 6 Chagres River villages (midbasin). None of these gave a positive response. No mouse protection tests were made on wild life.

There had been no indication of an epidemic in the province of Darien due to yellow fever, but from 1929 to 1937 there was a serious epidemic of smallpox in the Membrillo tribe of Indians which killed most of the tribe. The Rio Pirri tribe was almost wiped out by tuberculosis from 1929 to 1937. The tribes in the upper half of the Tucuti river had a serious influenza outbreak with a high death rate. I mention these things because word does reach the capital when things like this happen. However, yellow fever could easily escape notice if only sporadic cases appeared. There is an important interchange of people between the Atrata valley in Colombia and the Tuira valley in Panama. When the sugar mills are in operation in Colombia, there is but little labor migration,

but when the mills close, laborers move into Darien fruit, oil and lumber locations. In addition to this movement of labor, there is an average of about 20 people a month without papers who evade the law and cross the boundary, finally reaching Panama in small coastwise boats, and the same in true on the Atlantic coast.

During the months of November and December, 1948, five deaths occurred after one or two days of hospitalization in Santo Tomas Hospital, Panama City, from an acute febrile disease. No liver specimen was preserved from the first fatal case but after the fifth death it became evident that these cases strongly suggested yellow fever and on January 14, 1949, Drs. Elton and Herrera diagnosed yellow fever from the liver lesions found in two cases that died in December. These liver preparations were later brought to our laboratory for study and I agreed with their diagnosis. The delay is suspecting yellow fever was understandable because so many years had passed since there was any evidence of the occurrence of the disease in any part of the Republic. Furthermore, natives of Panama have always considered themselves highly resistant to the disease. Most of the doctors in charge of these cases had never had an opportunity to see yellow fever.

It is well to call attention to the fact that during World War II, there was a considerable development of secondary roads by the U.S. Army outposts considered necessary for the defence of the Panama Canal which resulted in some shift of rural population from such areas as the Azuero peninsula west of the Canal to formerly uninhabited and forested areas east of Panama City. Primary forest was cut, the land burned and cleared and family groups and small settlements became established. The felling of timber and the burning that followed exposed these people to mosquitoes of the forest canopy. A relatively small number came down with yellow fever and they were brought out to the highway for transportation to the hospital. Fortunately, all the highway settlements had been sprayed with 5% DDT in kerosene as part of the antimalaria campaign which incidentally wiped out *Aedes aegypti*. This, I believe, helped to control the spread of the disease. In August and September, 1949, three more fatalities from the disease occurred in the Buena Vista area. This is about half way across the transisthmian highway and near the upper end of an area of Gatun Lake known as the Rio Gatun basin.

In January, 1950, the first death due to yellow fever west of the Canal occurred on the Atlantic side about 12 miles west of Gatun Lake. On April 15, 1951, another fatal case was reported from Almirante Hospital well up the Atlantic coast. This man was a member of a construction gang surveying a line about 12 kilometers northwest of Almirante. This location is within 50 miles of the boundary line of Costa Rica. The disease has now appeared in epidemic form in two localities in Costa Rica, both of them on the Atlantic drainage slope. One is near Puerto Limon while the other is about 100 hundred miles up the slope.

At the request of the Yellow Fever Service, set up in 1949 jointly by the Health Department of the Panama Canal, the Medical Department of the Army and Navy, the Department of Public Health of the Republic of Panama and

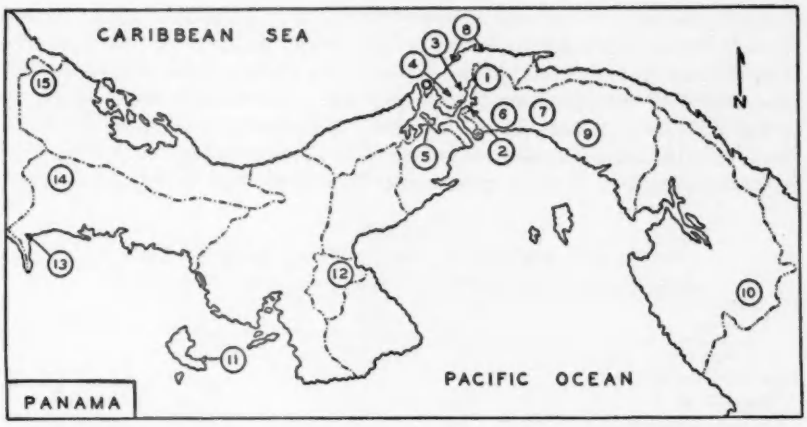
the Pan American Sanitary Bureau, the Gorgas Memorial Laboratory agreed to survey the arboreal forest mammals to determine the wild animal reservoir of jungle yellow fever in the Panama area. Blood specimens were taken from all animals at the site of the kills and at once placed in refrigeration. Later the serum was removed and forwarded to the Carlos Finlay Institute, Bogota, Colombia, for the application of the mouse protection test for immunity to yellow fever. Live animals caught or purchased during this period were tested by the Gorgas Memorial Laboratory.

After the 15 cc blood specimen was taken and placed in refrigeration, the animals were weighed and measured, the sex noted, and a note made as to whether it was an infant, juvenile, a young or very old adult. An autopsy was then performed and notes taken on the organs, the serous cavities, the teeth and the amount of fat storage. Blood films were made. A set of tissues was saved in 10-per cent formalin solution. Skins were taken from a few adult males and females of each species of animal and all skulls were denuded and saved. These were sent to Smithsonian Institution for scientific identification.

The regions surveyed are indicated on the accompanying map of Panama. Results of the mouse protection tests for immunity to yellow fever are shown in Table I, II, and III. Table IV gives the scientific identification of the animals tested.

In January, 1951, a report was received that monkeys were sick and dying near the village of Chorcha in Chiriqui Province, and that some of the people in the village were believed to have yellow fever. Chorcha is 40 minutes by jeep east of David on the highway to Panama City and from January 18 to 20 we searched the area. Two groups of howler monkeys were within hearing distance of the house from which some hospital cases had been received. A group of cebus monkeys was found but they escaped through the trees. A group of nine howlers was found and all of them killed and studied in the usual manner. The mouse protection tests were done by Dr. Enid de Rodaniche of the Gorgas Memorial Laboratory. The tests were all negative.

A common report that comes out of the jungle is that monkeys are sick and on the ground and many of them dying. Since 1915 I have had a chance to visit four regions from which such reports had been received and found none of them correct. Monkeys can be the victims of many diseases and when such reports are received they should be followed up and tests made to establish the diagnosis of yellow fever or to rule it out. Up to date, I have not had the privilege of finding a jungle epidemic of yellow fever in monkeys, yet there is much evidence in wild life surveys that monkeys have at some time during their lives had yellow fever and recovered. Our local species of monkeys while susceptible to the virus, do not suffer from the effects of yellow fever as the *rhesus* monkeys do. The monkeys we have collected on wild life surveys have required from one to three loads of buckshot to get them on the ground and even then some of them lodge in the trees and never fall. On two occasions following our leaving a jungle camp, natives have unexpectedly found groups of monkey carcasses and a report has come in that a serious disease is killing the monkeys. Even though



OUTLINE MAP OF THE REPUBLIC OF PANAMA WITH THE PROVINCES SHOWN IN DOTTED LINES

(Figures in circles indicate the regions surveyed)

1. Chagres River, Panama Province. Headwaters of the Chagres River, Pequeni branch. No monkeys taken here.

2. Chagres River, Panama Province. The eastern boundary of the Canal Zone runs through the center of the regions hunted. Juan Mina was used as the central camp.

3. Chagres River, Quebrada Jiral branch of Rio Palenque, Colon Province. A few miles north of the midbasin of the Chagres River.

4. Chagres River, Rio del Medio and Rio Patos, Colon Province. Two short streams that empty together into Rio Gatun just above and south of its mouth.

5. West bank of Gatun Lake, Panama Province. The shore line of Gatun Lake west of Barro Colorado Island.

6. Cabra River. Midway between the Chagres River and the Chepo-Bayano River, Panama Province.

7. Chepo-Bayano River, Panama Province. The midbasin was hunted including the branches Cañita, Majé, Mono, and Chulugandi.

8. Cacique-Palenque region on the Atlantic coast east of Colon. in Colon Province but near the San Blas islands.

9. Platanar River, Panama Province. The Platanar Branch of the Rio La Maestra which empties into the Pacific near Chiman and not far from the boundary of the Darien Province.

10. Tuira River, Darien Province. Headwaters of the river next to the boundary of Colombia.

11. Coiba Island (Penal Colony).

12. Azuero Peninsula (Santa Maria and Potuga), Herrera Province.

13. Rio Corotú, Chiriqui Province. Extends from the Pacific coast to the boundary of Costa Rica.

14. El Volcan-El Hato region, Chiriqui Province, near the boundary of Costa Rica. Elevation is 4,500 to 6,500 feet.

15. Almirante-Nievecita, Bocas del Toro Province, on the boundary line of Costa Rica behind the Atlantic seacoast.

every monkey carcass was without a head, the idea persisted that yellow fever was abroad.

At the request of Dr. Fred L. Soper, Director of the Pan American Sanitary Bureau, we were asked to make some surveys in southern Mexico similar to those we had made in Panama. Dr. Soper's office financed most of the expenses connected with the expedition. This survey was made in dry season, March 20 to April 25, 1951. It was desired to collect monkeys near Tampico and Vera Cruz but after consulting persons familiar with these areas, it was decided that the many industries in those regions had deforested the area to such a point

TABLE 1

Results of Mouse-Protection Tests for Immunity to Yellow Fever

Animals from eastern Panama (Canal Zone to Colombia), 1949–50

	NUMBER OF ANIMALS COLLECTED	NUMBER OF ANIMALS POSITIVE	PERCENT OF ANIMALS POSITIVE
Region surveyed:			
Tuira River	25	18	72.0
Cacique-Palenque	36	23	63.9
Cabra River	25	15	60.0
Chepo-Bayano River	33	16	48.5
Chagres River—Midbasin	36	10	27.7
Platanar River	46	22	47.8
Total	201	104	51.7
Species of animal:			
Howler monkeys	63	48	76.2
Whiteface monkeys	62	32	51.6
Marmosets	27	5	18.5
Night monkeys	9	2	22.2
Kinkajous-Olingos	17	3	17.6
Red spider monkeys	5	5	100.0
Black spider monkeys	7	6	85.7
Sloth, 3-toed	2	1	50.0
Sloth, 2-toed	2	0	0
Squirrel	2	0	0
Ocelot	1	1	100.0
Porcupine	1	0	0
Peccary	1	0	0
Anteater	1	0	0
Conejo pintado	1	1	100.0
Total	201	104	51.7

that monkeys could not persist in numbers. We finally decided on the state of Chiapas. The places selected were Palenque and the Chiapas Lumber Company at La Puerta about 60 kilometers beyond Cintalapa in the drainage basin of the Santa Maria river. Both places were on the Atlantic slope (see map). Forty monkeys were killed at Palenque and 37 behind Cintalapa.

The mouse protection tests were done by Dr. Rodaniche of the Gorgas Memorial Laboratory but all sera specimens were divided and half of each was sent

to the Serviço Nacional de Febre Amarela in Rio de Janeiro, Brazil, which reported that 74 or 12% were positive. Only 16 of the 40 blood specimens taken from monkeys in Palenque were considered by our laboratory to be in satisfactory condition to test and 2 of these, or 12.5 per cent, were positive. Both were from black howler monkeys. Of the 37 bloods taken from monkeys behind Cintalapa, 27 were in good shape for the tests and one of these was positive, or 3.7 per cent. This was a spider monkey. The howler monkeys from both locations were

TABLE 2

Results of Mouse-Protection Tests for Immunity to Yellow Fever

Animals from western Panama (Canal Zone to Costa Rica), 1950

	NUMBER OF ANIMALS COLLECTED	NUMBER OF ANIMALS POSITIVE	PERCENT OF ANIMALS POSITIVE
Region surveyed:			
El Volcan-El Hato	40	21	52.5
Coiba Island	40	15	37.5
Almirante-Nievecita	38	10	26.3
Azuero Peninsula	40	8	20.0
Rio Corutu	41	12	29.2
West Bank of Gatun Lake	25	2	8.0
Total	224	68	30.3
Species of animal:			
Howler monkeys	140	55	39.3
Whiteface monkeys	29	6	20.7
Red spider monkeys	11	2	18.2
Marmosets (squirrel)	13	0	0
Marmosets (Chiriqui)	10	1	10.0
Kinkajous-Olingos	13	3	23.1
Coatis	2	0	0
Opossums	2	1	50.0
Sloth, 3-toed	1	0	0
Porcupine	1	0	0
Raccoon	1	0	0
Squirrel	1	0	0
Total	224	68	30.3

identified by Smithsonian Institution as *Alouatta palliata pigra* and the spider monkeys as *Ateles geoffroyi vellerosus*.

Dr. Rodaniche has continued the survey for antibodies against yellow fever in the blood of live forest animals obtained by purchase. The intraperitoneal protection test in Swiss mice, with neurotropic virus, has been employed. A total of 91 mammals, including 75 monkeys and 16 other animals, have been examined. Of these three, or 3.3 per cent gave a positive result. The first of these was a juvenile male red spider monkey weighing 2325 grams, captured in the mountains northeast of Madden Dam and the town of Gatuncillo. The second

TABLE 3

Results of Mouse-Protection Test on Infant and Juvenile Monkeys Only

REGIONS	NUMBER EXAMINED	NUMBER POSITIVE	PERCENT POSITIVE	PERCENTAGE FOR TOTAL OF ALL AGES TESTED
Tuira River	4	1	25.0	72.0
Cacique-Palenque	3	2	66.6	63.9
Cabra River	3	1	33.3	60.0
Chepo-Bayano River	5	2	40.0	48.5
El Vocan-El Hato	5	2	40.0	52.5
Coiba Island	3	1	33.3	37.5
Chagres River	10	2	20.0	27.7
Rio Platanar	8	2	25.0	47.8
Almirante	4	0	0	26.3
Azuero Peninsula	9	1	11.1	20.0
Rio Corutu	1	0	0	29.2
West Bank Gatun Lake	2	0	0	8.0
Total	57	14	24.6	40.5

TABLE 4

Scientific identifications of animals collected (Smithsonian Institution report)

KIND OF ANIMAL	SCIENTIFIC NAME	LOCALITY WHERE TAKEN
Black howler monkey	*Alouatta pallita coibensis*	Coiba Island.
Black howler monkey	*Alouatta palliata trabeata*	Azuero Peninsula.
Black howler monkey	*Alouatta palliata palliata*	El Volcan.
Black howler monkey	*Alouatta palliata aequatorialis*	Rio Corutu and Nievecita.
Whiteface monkey	*Cebus capucinus capucinus*	Rio Platanar.
Whiteface monkey	*Cebus capucinus imitator*	Coiba Island, El Volcan and Almirante.
Red spider monkey	*Ateles geoffroyi panamensis*	Rio Corutu and Almirante.
Black spider monkey	*Ateles fusciceps robustus*	Rio Platanar.
Marmoset, Chiriqui	*Saimiri orstedii orstedii*	Rio Corutu.
Marmoset, squirrel	*Marikina geoffroyi*	Rio Tuira and Rio Platanar.
Kinkajou, isthmian	*Potos flavus isthmicus*	Rio Platanar.
Kinkajou, Chiriqui	*Potos flavus chiriquensis*	Chagres River (Rio del Medio) and Rio Corutu.
Opossum	*Didelphis marsupialis etensis*	El Volcan and Almirante.
Coati	*Nasua narica panamensis* Allen	Azuero Peninsula.
Sloth, 2-toed	*Choloepus hoffmani* Peters	Chagres River (Rio del Medio).
Sloth, 3-toed	*Bradypus grisens grisens*	Almirante (Nievecita).
Raccoon	*Procyon canarivorus panamensis*	El Volcan.
Squirrel	*Sciurus granatensis chiriquensis*	El Volcan.
Conejo pintado-paca	*Cuniculus paca virgatus*	Rio Platanar.

was a juvenile male black spider monkey weighing 2050 grams. It was captured in the Bayano River near the place where the human cases of yellow fever first appeared. The third was a young male anteater of the small species *Cyclopes*

didactylys dorsalis. It weighed 185 grams and was captured in the edge of the town of Curundu, Canal Zone.

In June of 1951, yellow fever virus was isolated for the first time during the current outbreak from a 25 year old male agricultural worker who was hospitalized with clinically typical symptoms of yellow fever. He recovered later. He was a native of Costa Rica working in the Province of Bocas del Toro, R. P., near the boundary of Costa Rica, when he became infected. By animal pathogenicity and immunological cross-reactions this strain has been identified definitely as yellow fever. The mouse test of serum taken at the time of his

discharge from Almirante Hospital was positive. The isolation of virus provided a valuable link in the chain of epidemiological data on the status of sylvan yellow fever in Panama. No virus has been recovered from any of the mosquitoes taken in the forest canopy survey although efforts are being continued in a Costa Rican area by Galindo and Trapido. No virus has been recovered to date from any of the monkeys or other animals.

In my opinion there is a wild life reservoir of yellow fever throughout Panama, Central America and southern Mexico. It is probable that a large percentage of cases in humans of rural areas of this geographic region follow a mild course and

go unrecognized. The occasional severe cases with fatal outcome are likely to occur remote from ready transportation, and fail to come to the attention of physicians with adequate hospital facilities, so that these too are not recognized. Although infective individuals might sporadically be transported into an urban area, the residual spraying of dwellings for the control of malaria, together with the direct attack carried on against *Aedes aegypti*, the urban vector, has removed the danger of any large scale urban epidemics such as those which materially affected the development of this area in the past. Vaccination and control or eradication of the *aegypti* mosquito provide an effective barrier to epidemics. All industries using large labor forces in forested regions should vaccinate their employees. Time is rapidly clearing away the primitive forest where the larger species of monkeys live but the small species and the kinkajou will thrive in second growth or bush country. At the present time the howler monkey, the spider monkey and the white face (cebus) are the animals showing the highest rate of positives to the mouse test.

IMMUNIZATION AGAINST SCRUB TYPHUS: DURATION OF IMMUNITY IN VOLUNTEERS FOLLOWING COMBINED LIVING VACCINE AND CHEMOPROPHYLAXIS

J. E. SMADEL[1], H. L. LEY, JR.[2], F. H. DIERCKS[3], P. Y. PATERSON[4], C. L. WISSEMAN, JR.[2] AND R. TRAUB[5]

From the Army Medical Service Graduate School and the Commission on Immunization of the Armed Forces Epidemiological Board, Washington, D. C.

During the last few years the problem of the therapy of scrub typhus has been conquered and control measures based on protection of the individual against the bite of the vector mite have been developed to a highly satisfactory state. Nevertheless, the protection of the persons operating under duress in scrub typhus infected areas remains a problem, since the satisfactory maintenance of anti-mite measures by the individual requires constant intelligent attention. Therefore, the search for a practical method for immunization against field exposure to scrub typhus has been continued. Despite the encouraging results obtained in several laboratories with non-infectious scrub typhus vaccines, field studies with these materials have provided no evidence of their value (see literature cited in reference 1). Such findings led to the study of the applicability of a method which induced infection by the inoculation of small numbers of living rickettsiae and then suppressed this below clinical levels by means of chemoprophylaxis. The first series of observations on this method (1) were encouraging and promised to provide a method which would be superior to that used several decades ago by the Japanese who induced moderately severe clinical disease in their immunity studies (2). The present report is concerned primarily with the duration of immunity in volunteers subjected to the combined procedure of inoculation with live vaccine and chemoprophylaxis; the resistance of such persons to a homologous and a heterologous strain of *Rickettsia tsutsugamushi* is examined in detail.

MATERIALS AND METHODS

Selection and care of volunteers. The majority of the volunteers employed in the present studies were persons who had served under our observation in previous field trials and each was known to have had either clinical disease with rickettsemia or clinical and serologic findings indicative of rickettsial infection (1, 3, 4). A number of new volunteers without history of scrub typhus who were selected on the same basis as before were also employed. Since the history of these volunteers as regards their previous experience with *R. tsutsugamushi* is

[1] M. D.
[2] Major, MC, U.S.A.
[3] 1st Lt., MSC, U.S.A.
[4] 1st Lt., MC, U.S.A.
[5] Lt. Colonel, MSC, U.S.A.

essential to the interpretation of the results of the present studies, data on this subject are given in appropriate places in the section on Results.

The procedures developed in earlier work in Malaya for the careful observation of volunteers and the care of patients were again employed (1, 5). Specific antibiotic therapy was used within 48 hours after the volunteers developed clinical scrub typhus; prompt therapeutic control of the disease was obtained here as in earlier studies (6). No supplementary doses of drug were given a week after the standard short course of treatment in order to prevent relapses since we were interested in the incidence of this phenomenon.

Preparation and use of living vaccine. Lyophilized vaccine containing living *R. tsutsugamushi* of the Gilliam strain was prepared and used as previously described (1, 7). In the present studies the Gilliam vaccine was made from the 112th yolk sac passage of rickettsiae. Lyophilized vaccine containing living scrub typhus organisms from the 68th yolk sac passage of the Karp strain was also employed in the present work. The history of this particular strain of Karp as well as certain of its characteristic properties are reviewed elsewhere (7). The Gilliam and Karp organisms differ not only in their antigenic constituents but also in their pathogenicity for mice, the former being of low and the latter of high mouse virulence. A third lyophilized vaccine was used primarily to test the immune state of one of the volunteers who had suffered scrub typhus $3\frac{1}{2}$ years before. This vaccine was prepared from strain C.P. 14 which had been through 6 passages in mice followed by 23 passages in the yolk sacs of embryonated eggs since its isolation from Volunteer C.P. 14 on the second day of his initial illness in 1948 (3, 8). The infective titers of the 3 vaccines, as determined in mice, at the time of their preparation a few months prior to use were as follows: Gilliam No. 6 infectious dose (ID_{50}) $10^{-6.5}$, lethal dose (LD_{50}) $10^{-1.7}$; Karp No. 14 ID_{50} $10^{-4.9}$, LD_{50} $10^{-4.1}$; C.P. 14 No. 2 LD_{50} $10^{-6.3}$. In these titrations in mice the infective dose was that which immunized the animal against subsequent challenge with a lethal amount of a mouse virulent strain; details of the procedure are presented elsewhere (7). Each time live vaccine was employed for the inoculation of volunteers the infectivity was re-determined by simultaneous titrations in mice. In estimating the quantity of viable rickettsiae which the volunteers actually received, the value for the infectious titer of the Gilliam material and for the lethal titers of the Karp and C.P. 14 materials were employed. For purposes of simplification, these results are expressed as mouse minimal infectious doses (mouse MID) irrespective of their origin.

Immediately prior to inoculation of volunteers, the lyophilized vaccine was rehydrated to the original volume and diluted in an appropriate salt solution containing 10 per cent inactivated serum from a selected volunteer without known history of scrub typhus or infectious hepatitis. One-tenth cc. of diluted vaccine containing the desired number of infectious doses was injected intradermally over the deltoid muscle.

Method of statistical evaluation. Where a statement of statistical significance occurs in the text, it is based on a comparison of two means, a type of analysis which lends itself readily to treatment with Student's t test. When an observed

difference is said to be significant, the calculated probability of such an event occurring by chance is 0.05 or less.

RESULTS

Resistance of volunteers to homologous and heterologous strains 1 year after vaccination with living R. tsutsugamushi. Twenty-two volunteers who had been inoculated intradermally the previous year with approximately 25 mouse MID of the Gilliam strain of *R. tsutsugamushi* returned for further study. The group consisted of equal numbers of volunteers whose original infection had been suppressed below clinical levels by chemoprophylaxis and those who had developed

TABLE 1

Summary of resistance of volunteers to homologous and heterologous reinfection

VOLUNTEER HISTORY	CHALLENGE STRAIN			
	GILLIAM		KARP	
	NO. VOLS. TESTED	CLINICAL DISEASE	NO. VOLS. TESTED	CLINICAL DISEASE
SUPPRESSED GILLIAM 14 MO. PREVIOUS	6	0	5	5
TREATED GILLIAM 14 MO. PREVIOUS	6	1	5	5
NO PREVIOUS SCRUB	8	8	7	7
SUPPRESSED KARP 1 MO. PREVIOUS	8	3	8	0
TREATED GILLIAM 2 MO. PREVIOUS	—	—	4	4
NO. PREVIOUS SCRUB	3	3	3	3

clinical disease prior to treatment. Volunteers of the two types were distributed in sub-groups and tested for resistance to intradermal inoculation with 125 mouse MID of the Karp strain or with 1000 mouse MID of the Gilliam strain. Information on the past histories as well as detailed findings in the present studies on these volunteers are presented in Appendix I.

The results of this test, which are summarized in Table 1, clearly show that volunteers resisted reinfection with the Gilliam strain but not with the heterologous Karp strain. This was true irrespective of whether the original infection had been clinically suppressed by prophylaxis or whether it had been followed by overt disease which was promptly terminated by specific antibiotic therapy. It may be noted that while all 8 of the control volunteers, without previous known

exposure to scrub typhus, developed clinical disease following inoculation with 1000 mouse MID of Gilliam, in contrast, only 1 of the previously vaccinated persons responded in a similar manner. All 10 of the previously vaccinated persons and all 7 of the control volunteers became ill following the injection of 125 mouse MID of Karp.

No information is available on the ultimate clinical severity of the disease in the vaccinated and unvaccinated groups since all volunteers were given specific therapy approximately 48 hours after onset of persistent fever, i.e., oral temperatures of 100°F. or above. Nevertheless, certain observations indicate that despite the 100 per cent attack rate of the volunteers inoculated with Karp, some resistance endured in members of the vaccinated group. Thus, 4 of the 10 vaccinated persons failed to develop eschars at the site of inoculation whereas all 7 of the controls displayed this lesion. Furthermore, the average incubation period in the controls who received the Karp challenge was 10.1 days in contrast to 13.6 days in the vaccinated group. Moreover, the incubation period in the subgroup whose disease had been suppressed following original vaccination was 12.6 days while in the other sub-group which had required treatment the incubation period was 14.6 days. The differences in incubation periods, even those between the vaccinated sub-groups, are statistically significant despite the small size of the groups. Similarly, examination of the maximum temperatures recorded for the volunteers in the first 2 days of illness prior to initiation of therapy gives further evidence of heterologous resistance. The mean maximum temperature observed in the vaccinated group was 102.9°F. while that noted in the control group was 104.1°; this difference is statistically significant. In spite of these indications of some resistance, it will be noted in Appendix I that the relapse rate in the vaccinated and unvaccinated persons who received Karp was essentially the same, viz., 2 of 10 and 2 of 7. Since clinical scrub typhus in our experience almost invariably has been associated with rickettsemia, the demonstration of this finding in all of the 17 volunteers receiving Karp added nothing to the interpretation of the clinical observations except laboratory diagnostic proof of the etiology of the episode.

Ancillary evidence of resistance of the volunteers to challenge with the homologous Gilliam strain supported the clinical observations, namely, that 1 of the 12 developed overt disease. This single individual was the only member of the vaccinated group who displayed an eschar while all 8 of the controls did so. Two other vaccinated volunteers in the group (Nos. 607 and 658), who had mild complaints but were afebrile during the period when the controls were developing disease, were also found to have rickettsemia; however, these symptoms disappeared within several days without treatment and the volunteers remained ambulatory.

Resistance of volunteers to homologous and heterologous strains 1 to 2 months after vaccination. Sixteen volunteers were available who had been immunized recently by the combined procedure, i.e., intradermal inoculation of 60 mouse MID of Karp followed by intermittent chloramphenicol chemoprophylaxis for 3 or 4 weeks (9). These were divided into 2 groups and inoculated intradermally

with 90 mouse MID of the Karp strain or with 100 mouse MID of the Gilliam strain. The results of this test are given in detail in Appendix II along with those obtained on normal volunteers who served in the control groups. In addition, the Appendix contains immunological data on a group of volunteers who had served 2 months previously as controls in the test mentioned in the preceding paragraph and who had received specific therapy after clinical scrub typhus had developed. Table I summarizes the incidence of clinical disease in this short term experiment.

It is apparent from Table1 and Appendix II that the vaccinated volunteers in the present test were solidly immune to challenge with the homologous Karp strain 1 month after the original inoculation. They developed neither constitutional signs of illness nor eschars. Since tests for rickettsemia were limited to those volunteers who presented clinical evidence of infection, this procedure was not employed in the group under discussion. In contrast to this solid immunity to the homologous organism, 3 of the 8 Karp immune volunteers developed scrub typhus when challenged with 100 mouse MID of the heterologous Gilliam strain 1 month after vaccination. The incubation period was prolonged, i.e., 14 to 19 days, average 17.0, in contrast to that in the non-immune control group, i.e., 11 to 12 days, average 11.7. This difference in means is statistically significant. None of the vaccinated volunteers displayed eschars while all members of the control group did so.

Of particular interest in the present test was the response of the Gilliam convalescent volunteers who were inoculated with 90 mouse MID of heterologous Karp strain approximately 2 months after infection with the Gilliam organism. It will be recalled that each of these 4 persons had been permitted to develop clinical disease following the original inoculation and had suffered 3 to 6 days of fever (see Appendix I). Each of these persons displayed signs of clinical scrub typhus beginning on the 18th or 19th day after the Karp inoculation; these signs included severe headache, mild malaise, slight conjunctival injection and low-grade fever. One volunteer (No. R-5-4) reached 100.4°F. on one occasion, the others never went above 100.0°F. and this was recorded for only 1 or 2 days. The volunteers remained ambulatory and one insisted on continuing heavy labor in a neighboring tin mine. Despite the mildness of the illness, therapeutic doses of antibiotic were given on the 2nd day after onset of fever; it was our opinion that this therapy might have been omitted but it was given primarily for administrative reasons. Despite the constitutional signs, none of the vaccinated volunteers in this group developed eschars.

Duration of immunity to homologous strain. Additional information on the duration of immunity to the homologous infecting strain became available as a result of studies on Volunteer C.P. 14. This individual had participated in the first Malayan field trial 3½ years before and had contracted scrub typhus as a result of being bitten by infected mites (8). Volunteer C.P. 14 and a person who had no previous history of scrub typhus were injected intradermally with 20 mouse MID of the C.P. 14 strain of rickettsia. The control volunteer, C-27, developed the usual disease after an incubation period of 10 days while Volun-

teer C.P. 14 remained entirely asymptomatic during the period of observation (see Appendix I).

These findings supplement the previous observations on Volunteer V-10 (5) who, like C.P. 14, was solidly immune to challenge with his homologous strain 3½ years after the original disease.

Duration of immunity following multiple experiences with living R. tsutsugamushi. Studies were undertaken concurrently with those just described to determine the duration of immunity in persons who had had two or more experiences with scrub typhus, the last being some 15 months earlier with the Gilliam strain. Nine volunteers from field trials 1 and 3 (3, 4, 8) who had had rickettsemia during the course of clinical or suppressed disease which followed exposure to infected mites, had been inoculated intradermally 15 months earlier with 10 to 100 mouse MID of the Gilliam strain (5). Six of these volunteers had remained well following the initial test for immunity while 3 (C.P.-12, 111 and 150) had developed another attack of scrub typhus. Those who were resistant in the previous test were inoculated with 1000 mouse MID of Gilliam while those who had been susceptible 15 months before were injected with 125 mouse MID of Karp. These volunteers were inoculated with aliquots of the challenge material given concurrently to participants in another study; the same normal persons served as control for the two investigations. The results obtained with the current group are summarized in Appendix III while the observations on the control volunteers are given in Appendix I.

Five of the 6 who had failed to develop clinical disease following the previous inoculation of Gilliam organisms remained well after receiving the current injection of viable Gilliam organisms. One member of the group developed clinical scrub typhus with an eschar and with onset of fever 14 days after inoculation. The normal volunteers who had received portions of this same inoculum became ill after an average incubation period of 10.6 days. All 3 of the volunteers who had developed scrub typhus as a result of their Gilliam inoculation 15 months before again experienced clinical scrub typhus when injected with 125 mouse MID of Karp rickettsiae. None of these showed an eschar but all developed fever in 15 to 17 days. It was the clinical impression that the disease in these 3 was milder than in the controls, whose average incubation period was 10.1 days. Nevertheless, specific therapy was given on the 2nd febrile day with the usual beneficial results.

Local reactions at the site of inoculation. The cutaneous lesions which appeared at the sites of inoculation of volunteers in the present control groups who received Gilliam or Karp, followed the same pattern observed in earlier studies when Gilliam alone was used (5). The initial response, a small erythematous lesion which appeared within a few hours and gradually subsided over the next few days, was a non-specific reaction to the yolk sac material in the living vaccine. This was indicated by two lines of evidence, (a) solidly immune volunteers who failed to develop primary eschars or clinical disease showed the same initial reaction, and (b) 10 normal persons displayed similar reactions when inoculated with normal yolk sac material processed in the same manner as the infected

yolk sac which constituted the living vaccine. The specific primary lesion began about the 5th day after inoculation as a small papule. This progressed in size and developed a pale ischemic center within the next 5 days at which time the patient developed fever and constitutional signs. In most of the volunteers in the control group the typical black necrotic eschar showed itself within the next day or so at about the time treatment was instituted. Healing occurred during the week following defervescence.

In the earlier studies on the resistance of patients recovered from scrub typhus, it was observed that all who developed clinical disease following inoculation displayed typical eschars, while those who were resistant failed to have the primary lesion (5). While this generality remained true in portions of the current work, attention should be drawn to the 2 groups who had been infected with Gilliam 1 year or 2 months before inoculation with Karp, see Appendices I and II. All members of both groups developed clinical disease as a result of the Karp inoculation yet none of the latter group and only about half of the former showed primary lesions.

Among the partially immune persons in the present study who had delayed incubation periods following challenge, the same general time relationship between the appearance of eschar and fever was observed as in the infected normal controls, namely, the eschar became recognizable several days before the onset of fever.

Low incidence of positive Weil-Felix tests in re-inoculated volunteers. Only about 40 per cent of the volunteers who developed illness following reinoculation with *R. tsutsugamushi* after 1 year displayed a significant (fourfold) rise in titer of OX-K agglutinins, namely, 4 of 11 persons (see Appendices I and II). This is in contrast to the observations on the control volunteers in the present studies, 80 per cent of whom developed positive Weil-Felix reactions, namely 18 of 22. Only 1 of the 7 volunteers who became ill when re-inoculated with a heterologous strain 1 to 2 months after the initial procedure showed a Weil-Felix response. The over-all incidence of positive serological findings was even lower among the volunteers who did not become ill following re-inoculation with living rickettsiae. Thus, 2 of 12 in this category who were tested after 1 year showed a response whereas all 13 of those tested after 1 or 2 months gave negative results.

In general, the present observations agree with those on volunteers in our earlier studies who had a second experience with *R. tsutsugamushi* (5). Furthermore, it is worth comparing the present data with those of Murray and his coworkers (10) on the low incidence of positive Weil-Felix reactions in Brill's disease which is now almost universally regarded as a second attack of epidemic typhus.

DISCUSSION

The present studies supplement earlier observations on the duration of immunity to *R. tsutsugamushi* in treated patients (5); moreover, they indicate that the level of immunity in persons who have experienced a suppressed infection elicited by living vaccine plus chemoprophylaxis is of the same order of magni-

tude as that in such recovered patients. The data continue to indicate that immunity to the homologous strain is almost complete and usually persists for at least several years. On the other hand, both recovered treated patients and volunteers with suppressed disease are susceptible to reinfection accompanied by clinical manifestations when tested after a few months with a heterologous strain.

Resistance to the heterologous strain was not completely absent even though volunteers developed clinical disease following inoculation. This maintenance of some resistance was indicated by the low incidence of eschar formation, the prolongation of the incubation period, and the milder fever during the first few days of illness before the disease was terminated by specific therapy.

The exact time at which immunity to the heterologous strain begins to be lost cannot be stated definitely from the present observations. However, the data summarized in Appendix II show that 5 of 8 volunteers originally infected with Karp were solidly resistant when tested with Gilliam 1 month after inoculation. Furthermore, volunteers infected originally with Gilliam were uniformly susceptible to the Karp strain inoculated 2 months later. Whether these findings reflect differences in the antigenic structure and virulence of the two organisms employed or whether they indicate the waning of heterologous immunity between the 1st and 2nd month remains undecided.

Some idea of the progressive loss of resistance over a period of almost a year may be gained by comparing the group containing Volunteers 603 through 656 in Appendix I with the group containing Volunteers R-5-1 through R-5-4 in Appendix II. Both groups consisted of persons with treated scrub typhus caused by the Gilliam organism and each was tested for resistance to approximately 100 mouse MID of Karp organisms at an interval of 1 year and at 2 months, respectively, after original inoculation. Although the Karp inoculations were done at different times, the disease induced in the control volunteers in each instance was almost identical. For example, the average incubation periods in the 2 groups were 10.1 and 10.3 days. Two of the 5 volunteers tested at 1 year developed eschars and the average incubation period before onset of fever was 14.6 days. In contrast, none of the 4 who received their Karp material at 2 months developed eschars and the average incubation period was 18.3 days. Furthermore, in the experiment of short duration, the febrile response was minimal and may actually have terminated before therapy could be instituted. The febrile response of the volunteers in the 1 year experiment was greater and lasted until it would have been expected to disappear as a result of therapy.

Our experience like that of our predecessors has repeatedly emphasized the difference in the incidence of eschar formation in Asians and Caucasians who contract scrub typhus in South East Asia (8, 11, 12). The present studies provide definitive data which support our earlier hypothesis (8) that partial immunity of the human host might prevent the appearance of the local primary lesion even though generalized disease might occur. Thus, none of the partially immune volunteers in the short term study summarized in Appendix II displayed eschars and not all of the susceptible but still slightly resistant volunteers

in the long term experiment summarized in Appendix I showed this lesion, whereas all 22 volunteers selected for controls developed eschars at the site of inoculation. The occurrence of the primary lesion in the patient with scrub typhus, be he Asian or Caucasian, probably depends upon both the degree of immunity of the individual and the size of the infecting dose. One wonders whether the incidence of scrub typhus among the rural Malayan population might be rather high during childhood or adolescence and whether exposures might not continue to occur and produce occasional mild illnesses which are not recognized as classical scrub typhus. Should such a situation exist, it would supply an explanation for the low incidence of eschars among Malayans who develop scrub typhus as a result of the exposure during normal occupational duties (3, 11) or supervised exposure in hyperinfected areas (8).

If scrub typhus infections are as ubiquitous among the Malayans as postulated, then one must account for the high incidence of infection, i.e., 75 per cent, among volunteers in 2 of the field trials carried out under conditions which would simulate natural exposure. Furthermore, one must explain the essential absence of immunity in Malayans inoculated intradermally with living *R. tsutsugamushi*. We would suppose that differences in the two factors mentioned above, namely, resistance of the host and infecting dose, could provide enough modification of individual responses to reconcile the two divergent observations. In this discussion we have avoided the matter of antigenic variations among strains of rickettsiae since these are so numerous and extensive that it would be essentially impossible to evaluate them. It is worth pointing out, however, that the Karp and Gilliam strains differ antigenically from one another and from 8 other strains of *R. tsutsugamushi* which were recovered from a field near Kuala Lumpur where volunteers were exposed (13, 14).

Present observations which indicate the short duration of solid immunity to heterologous strains are in general accord with the earlier work of the Japanese investigators (15). Kawamura and his associates who injected volunteers with the moderately virulent Pescadores strain and shortly after recovery tested their resistance to the more virulent Niigata strain found that 4 of 20 developed mild disease. Our attack rates were higher since 4 of 4 and 3 of 8 developed signs of illness when tested for immunity with a heterologous strain 1 to 2 months after the original inoculation. It is possible that the Pescadores disease which lasted 2 to 3 weeks provided a more durable heterologous immunity than the short or absent clinical illness which was encountered in our treated volunteers or in those with chemoprophylactically suppressed infection. The important conclusion to be drawn from the Japanese work, our own earlier studies, and the present investigation is that resistance to infection with heterologous strains of *R. tsutsugamushi* is lost within a matter of months.

The present report is not concerned primarily with the evaluation of the usefulness of the procedure of immunization by inoculation with live vaccines followed by suppressive chemoprophylaxis in the field control of scrub typhus. While some effort toward such an evaluation is made in another article of this series (9), it may be stated here that we have been discouraged by the short

duration of solid heterologous immunity under the conditions of the present study.

SUMMARY

1. The immunity which developed in volunteers as a result of suppressed infection with *R. tsutsugamushi*, obtained by inoculation of living vaccine followed by a course of chloramphenicol chemoprophylaxis, was essentially the same as that displayed by volunteers who, following inoculation, experienced overt disease which was terminated by specific therapy.

2. Immunity to the homologous strain of rickettsiae persisted at a high level in most persons for at least 1 year and in some for longer periods.

3. Resistance to infection with heterologous strains of *R. tsutsugamushi* was of a transient nature. Even within a month after inoculation with one strain, an appreciable number of persons became ill following injection with a heterologous strain. By the end of a year, all were again susceptible to heterologous infection. During the period of waning immunity, the disease which resulted from the heterologous strain was modified from the classical picture. Indeed, the illness observed in the test shortly after recovery was so mild that it would probably not have been recognized as scrub typhus if rickettsemia had not been demonstrated. On the other hand, the disease which resulted when the individuals were tested after 1 year generally presented the typical picture of scrub typhus.

ACKNOWLEDGEMENT

The authors wish to thank the volunteers for their cheerful cooperation through all the minor vicissitudes of their service. Grateful acknowledgement is made of the assistance extended by Dr. J. W. Field, Director of the Institute for Medical Research, Kuala Lumpur, and the entire staff of the Institute, and by the Government of the Federation of Malaya, particularly the Medical Service.

REFERENCES

In future issues of this Journal, references will be cited by date and not by number.

1. Smadel, J. E., Ley, H. L., Jr., Diercks, F. H., Traub, R., Tipton, V. J., and Frick, L. P., Immunization against scrub typhus. I. Combined living vaccine and chemoprophylaxis in volunteers, *Am. J. Hyg.* **53:** 317–325, 1951.
2. Kawamura, R., Kasahara, S., Toyama, T., Nishinarita, F., and Tsubaki, S., On the prevention of tsutsugamushi. Results of preventive inoculations for people in the endemic region, and laboratory tests with the Pescadores strain, *Kitasato Arch. Exper. Med.* **16:** 93–109, 1939.
3. Smadel, J. E., Woodward, T. E., Ley, H. L., Jr., and Lewthwaite, R., Chloramphenicol (Chloromycetin) in the treatment of tsutsugamushi disease (scrub typhus), *J. Clin. Invest.* **28:** 1196–1215, 1949.
4. Smadel, J. E., Traub, R., Frick, L. P., Diercks, F. H., and Bailey, C. A., Chloramphenicol (Chloromycetin) in the chemoprophylaxis of scrub typhus (tsutsugamushi disease). III. Suppression of overt disease by prophylactic regimens of four-week duration, *Am. J. Hyg.* **51:** 216–228, 1950.
5. Smadel, J. E., Ley, H. L., Jr., Diercks, F. H., and Traub, R., Immunity in scrub typhus: resistance to induced reinfection, *A.M.A. Arch. Path.* **50:** 847–861, 1950.

6. Bailey, C. A., Ley, H. L., Jr., Diercks, F. H., Lewthwaite, R., and Smadel, J. E., Treatment of scrub typhus: evaluation of chloramphenicol, aureomycin, terramycin and para-aminobenzoic acid, *Antibiotics* **1:** 16–30, 1951.
7. Jackson, E. B., and Smadel, J. E., Immunization against scrub typhus. II. Preparation of lyophilized living vaccine, *Am. J. Hyg.* **53:** 326–331, 1951.
8. Smadel, J. E., Traub, R., Ley, H. L., Jr., Philip, C. B., Woodward, T. E., and Lewthwaite, R., Chloramphenicol (Chloromycetin) in the chemoprophylaxis of scrub typhus (tsutsugamushi disease). II. Results with volunteers exposed in hyperendemic areas of scrub typhus, *Am. J. Hyg.* **50:** 75–91, 1949.
9. Ley, H. L., Jr., Diercks, F. H., Paterson, P. Y., Smadel, J. E., Wisseman, C. L., Jr., and Traub, R., Immunization against scrub typhus. IV. Living Karp vaccine and chemoprophylaxis in volunteers. In preparation.
10. Murray, E. S., Baehr, G., Shwartzman, G., Mandelbaum, R. A., Rosenthal, N., Doane, J. C., Weiss, L. B., Cohen, S., and Snyder, J. C., Brill's disease. I. Clinical and laboratory diagnosis, *J. Am. M. Assoc.* **142:** 1059–1066, 1950.
11. Lewthwaite, R., and Savoor, S. R., The typhus group of diseases in Malaya. Part VII: The relation of rural typhus to the tsutsugamushi disease (with special reference to cross-immunity tests), *Brit. J. Exp. Path.* **17:** 448–460, 1936.
12. Kouwenaar, W., and Wolff, J. W., Rickettsia infections in Sumatra, *in* Proceedings of the Sixth Pacific Science Congress of the Pacific Science Association, University of California Press, 1942.
13. Bennett, B. L., Smadel, J. E., and Gauld, R. L., Studies on scrub typhus (tsutsugamushi disease). IV. Heterogeneity of strains of R. tsutsugamushi as demonstrated by cross-neutralization tests, *J. Immun.* **62:** 453–461, 1949.
14. Miesse, M., Diercks, F. H., and Danauskas, J. X., Strain differences among Rickettsia tsutsugamushi, *Bact. Proc.* 1950, pp. 90–91.
15. Kawamura, R., Ito, T., Nakamura, R., Kamimura T., and Sato, I., On the prevention of tsutsugamushi disease including a new method of fever therapy. *Kitasato Arch. Exper. Med.* **47:** 75–98, 1937.

APPENDIX I

Resistance of volunteers to reinfection with homologous and heterologous strains after one year

VOL. NO.	TYPE OF PREVIOUS CONTACT	INOCULUM		RESULTS OF INOCULATION							
		STRAIN	MOUSE INFECTIOUS DOSES	CLINICAL DISEASE	ESCHAR	ONSET OF FEVER, DAYS AFTER INOC.	RICKETTSEMIA	RELAPSE	TOTAL DURATION FEVER DAYS	W.F	OX-K
										PRE.	POST
607	GILLIAM CLINICAL DISEASE	GILLIAM	1000	0	0		+			20	20
608+				+	+	10	+	0	4	20	40
615				0	0		ND			20	20
646				0	0		ND			80	80
655				0	0		ND			80	20
658				0	0		+			20	80
610	GILLIAM V-C SUPPRESSED DISEASE	GILLIAM	1000	0	0		ND			20	40
628				0	0		ND			20	20
634				0	0		ND			40	80
636				0	0		ND			80	40
637				0	0		ND			80	20
639				0	0		ND			40	80
R-5-1	NONE	GILLIAM	1000	+	+	11	+	0	3	40	160
R-5-2				+	+	11	+	+	5	20	160
R-5-3				+	+	11	+	0	4	40	640
R-5-4				+	+	10	+	0	3	20	40
R-5-5				+	+	10	+	0	3	40	160
R-5-6				+	+	11	+	0	3	0	160
R-5-7				+	+	11	+	0	4	0	80
R-5-8				+	+	10	+	0	4	20	40
603	GILLIAM CLINICAL DISEASE	KARP	125	+	0	16	+	0	3	20	320
616+				+	0	15	+	0	3	20	40
619				+	+	13	+	+	6	40	160
654				+	0	14	+	0	3	0	40
656				+	+	15	+	0	3	80	80
620	GILLIAM V-C SUPPRESSED DISEASE	KARP	125	+	+	13	+	0	3	20	160
622				+	+	12	+	0	4	0	20
623				+	0	14	+	0	3	40	20
629				+	+	12	+	0	4	40	80
626				+	+	12	+	+	6	40	80
R-5-9	NONE	KARP	125	+	+	10	+	0	4	20	160
R-5-10				+	+	10	+	0	4	40	160
R-5-11				+	+	10	+	0	6	40	160
R-5-12				+	+	10	+	+	6	40	1280
R-5-13				+	+	11	+	0	3	40	80
R-5-14				+	+	10	+	+	4	40	640
R-5-15				+	+	10	+	0	4	20	320
CP-14	CLINICAL DIS.	CP-14	20	0	0					40	20
C-27	NONE			+	+	10	+	+	5	40	320

VOLUNTEERS PREVIOUSLY INFECTED WITH KARP WERE TESTED FOR IMMUNITY 14 TO 15 MONTHS LATER. VOLUNTEER CP-14 WAS CHALLENGED 40 AFTER ORIGINAL ILLNESS

+ VOLUNTEERS 608 AND 616 WERE CHALLENGED ONE MONTH AFTER THEIR ORIGINAL ILLNESS AND WERE RESISTANT TO 9 MID50 GILLIAM STRAIN (3)
ND INDICATES NOT DONE

APPENDIX II

Resistance of volunteers to reinfection with homologous and heterologous strains after one month

VOL. NO.	TYPE OF PREVIOUS CONTACT	INOCULUM		RESULTS OF INOCULATION							
		STRAIN	MOUSE INFECTIOUS DOSES	CLINICAL DISEASE	ESCHAR	ONSET OF FEVER, DAYS AFTER INOC.	RICKETTSEMIA	RELAPSE	TOTAL DURATION FEVER DAYS	W.F	OX-K
										PRE	POST
BB-1-4	KARP V-C	KARP	90	0	0		ND			40	40
BB-1-5	SUPPRESSED			0	0		ND			40	80
BB-1-6	DISEASE			0	0		ND			160	80
BB-2-4				0	0		ND			160	80
BB-2-5				0	0		ND			160	160
BB-2-6				0	0		ND			320	320
BB-3-3				0	0		ND			20	20
BB-3-4				0	0		ND			40	80
R-5-1	GILLIAM	KARP	90	+	0	18	+	0	2	20	80
R-5-2	CLINICAL			+	0	19	+	0	1	20	80
R-5-3	DISEASE			+	0	18	+	0	2	640	320
R-5-4				+	0	18	+	0	1	20	20
C-21	NONE	KARP	90	+	+	10	+	+	6	20	5120
C-22				+	+	10	+	0	3	0	80
C-23				+	+	11	+	0	3	0	40
BB-1-1	KARP V-C	GILLIAM	100	0	0		ND			20	40
BB-1-2	SUPPRESSED			0	0		ND			2560	640
BB-1-3	DISEASE			+	0	14	+	0	3	20	20
BB-2-1				0	0		ND			160	160
BB-2-2				+	0	18	+	0	1	80	160
BB-2-3				+	0	19	+	0	3	40	80
BB-3-1				0	0		ND			0	160
BB-3-2				0	0		ND			80	20
C-24	NONE	GILLIAM	100	+	+	12	+	0	3	20	20
C-25				+	+	11	+	0	4	0	1280
C-26				+	+	12	+	0	3	0	80

VOLUNTEERS PREVIOUSLY INFECTED WITH KARP WERE TESTED FOR IMMUNITY ONE MONTH LATER; THOSE PREVIOUSLY INFECTED WITH GILLIAM, TWO MONTHS LATER. ND INDICATES NOT DONE

APPENDIX III

Resistance to reinfection in volunteers having multiple contacts with R. tsutsugamushi

VOL. NO.	TYPHUS HISTORY			CHALLENGE 1951								
	INITIAL DISEASE FOLLOWING FIELD EXPOSURE 1948 OR 1949		GILLIAM CHALLENGE 1950	INOCULUM		RESULTS OF INOCULUM					W.F	OX-K
	RICKETTSEMIA	HOSPITALIZED	CLINICAL DISEASE	STRAIN	MOUSE INFECTIOUS DOSES	CLINICAL DISEASE	ESCHAR	ONSET OF FEVER DAYS AFTER INOC.	RICKETTSEMIA	TOTAL DURATION FEVER DAYS	PRE	POST
CP-10	+	YES	0	GILLIAM	1000	0	0		ND		40	20
44	+	YES	0			+	+	12	+	4	20	80
114	+	NO	0			0	0		ND		20	80
115	+	YES	0			0	0		ND		40	20
134	+	YES	0			0	0		ND		40	40
144	+	NO	0			0	0		ND		40	40
CP-12	+	YES	+	KARP	125	+	0	17	+	2	0	0
111	+	NO	+			+	0	15	+	2	80	80
150	+	YES	+			+	0	15	+	4	20	40

SEE APPENDIX I FOR RESPONSE OF CONTROL VOLUNTEERS WHO RECEIVED PORTIONS OF SAME CHALLENGE INOCULUM
ND INDICATES NOT DONE

THE INFLUENCE OF THE NATIONAL MALARIA SOCIETY ON MALARIA ERADICATION[1]

JUSTIN M. ANDREWS

Communicable Disease Center, Public Health Service; Federal Security Agency, Atlanta, Georgia

Of the 29 "charter" members (1, 2) of the National Malaria Committee, 3 are still alive. Since the Committee's founding less than a score of years after Ross discovered the key to malaria transmission, they have witnessed the conquest of malaria, and the resultant relief from its baneful effects upon human existence in this land. This transition is memorable in the annals of communicable disease control, but as some anonymous author has said, "What's past is soon forgotten and in the forgetting loses all substance."

It was estimated in 1916—the year the Committee was started—that not less than 1,000,000 cases and 15,000 deaths due to malaria occurred in the continental United States.[2] (1) Malaria was not the scourge then that it had been in pioneering days, but there were still plenty of areas in the southeastern coastal and fluvial plains where crops could not be made on good bottom land because of the ague, where wishful investors passed by promising industrial sites because of the local reputation for fever, where many of the white rural schools had "chillin' " beds, and where all country stores carried quinine and bewildering assortments of patent antimalarials. In 1919, Dr. Henry R. Carter estimated (3) that malaria cost this country about $100 million a year. As late as 1938, Dr. L. L. Williams, Jr., gave his reasons (4) for believing that the annual economic loss approximated $500 million. Even making generous allowance for the natural enthusiasm of these protagonists for bigger and better malaria control budgets, it is evident that malaria absorbed many millions of dollars each year in a period when dollars were harder to come by and were worth much more than they are at present.

[1] The President's Address, delivered at the 34th Annual Meeting of the National Malaria Society, Chicago, Illinois, November 15, 1951.

[2] This is a far cry from what was actually reported—but the estimate is probably closer to the truth than the official returns which were as follows. According to Notifiable Diseases, Public Health Reports, 32: 1506–1531, 1917, some 175,845 cases were reported from 14 States: Alabama, California, Colorado, Kansas, Louisiana, Maryland, Massachusetts, Minnesota, Mississippi (reported 158,751 cases), New Jersey, Ohio, Pennsylvania, South Carolina, and Virginia.

A total of 3,153 deaths from malaria were certified from all the States above except Colorado and Ohio from which no death data were furnished. This list did not include the notably malarious States of Arkansas, Florida, Georgia, Kentucky, Missouri, North Carolina, Oklahoma, Tennessee, and Texas. Mortality reports from the Registration Area for 1916, 26 States (not including Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, Oklahoma, Tennessee, and Texas), the District of Columbia, and 35 cities in Alabama, Delaware, Florida, Georgia, Illinois, Louisiana, Mississippi, Nebraska, North Dakota, Oregon, and West Virginia, listed 1,898 malaria deaths from the States and 277 from the cities. (Mortality Statistics, 1916; 17th Annual Report, Department of Commerce, Bureau of the Census, Washington: Government Printing Office, 1918. pp. 208–232).

Today, the erstwhile miasmatic Tennessee River Valley is a resort area. Agricultural practices have changed drastically throughout the Southeast during the intervening years, but I doubt that a single acre now remains fallow on account of malaria. Health officers no longer receive from prospective manufacturers demands for facts and figures regarding the salubrity of their jurisdictions, especially as far as paludism is concerned. The dusty bottles of chill tonics occupy only a small space on the store shelves these days and are used, according to the storekeepers, "mostly for teething babies." Indeed, the signs advertising these products, which used to adorn so conspicuously fence posts and the sides of barns along the country roads throughout the South, are now hard to find. Present Federal-State expenditures for malaria surveillance and prevention now amount to only about a half million dollars per year. It seems unlikely that the costs of medical care and income lost during sickness from reported malaria—even including the unproved cases—would amount to another half-million of our depreciated dollars. The latest available mortality data are for 1949. They tally 118 malaria deaths for the Nation, but the testimony of epidemiologists in the Southeastern States where almost all of the deaths occurred suggests that few if any were really caused by malaria and nothing else. In 1950, some 2,227 cases were reported as against 4,231 for the previous year. However, the figure for 1951 will probably be two or three times as high, the difference being military cases introduced mainly from Korea (5). Nevertheless, careful investigation and appraisal to exclude unproven, imported, transfused, and relapsing infections have revealed so few instances of parasite-positive malaria, and these so scattered and unrelated geographically, it is difficult to imagine how transmission could have occurred in this country during recent years (6).

Thus a disease important enough only 35 years ago to beget an organization dedicated to its elimination seems to have vanished from the national scene as an endemic infection. This has been accomplished presumably as a result of a favorable combination of adventitious factors and of purposeful efforts on the part of many people. It is the aim of this essay first to remind you of some of the means by which members of the National Malaria Committee and the National Malaria Society have been instrumental in promoting and achieving this end, and secondly, how this same group, no matter what its future designation, can continue to influence the maintenance of malaria eradication in this country and the extension of this beneficent condition abroad.

As you all know, the National Malaria Committee owes its beginning to the crusading efforts of Dr. Frederick L. Hoffman, Statistician for the Prudential Life Insurance Company. He proposed the Committee as the centralizing agency in his plan to eradicate malaria from the Western Hemisphere and particularly from this country. Thus he was responsible not only for the creation of the Committee but for establishing its goal—malaria eradication. I must confess that until within the last year, I had assumed, based upon a cursory reading of his first two malaria papers, (1, 2) that he used the term "eradication" more journalistically than literally. But having reread these articles more recently, and his other publications on malaria as well, I am sure that Dr. Hoffman had in mind nothing less than the total elimination of the disease. He was firmly

convinced that this could be brought about only by the wholesale extermination of its anopheline vectors as is indicated in the following quotation from a progress report read before the New Jersey Mosquito Extermination Association in 1918: (7) "... Although more directly concerned with malaria eradication, I have long since come to realize the obvious practical importance of any and all mosquito-extermination efforts. Personally, I am unequivocally of the opinion that the former will never be achieved without the latter, and that no matter how many different methods of malaria prophylaxis may be suggested or advocated, in its final analysis any effort must fail to rid the community of malaria if the Anopheles mosquitoes are not entirely done away with. You may sterilize your carriers; you may practice quinine prophylaxis; you may screen your houses; you may oil your ponds; you may spend hundreds of thousands of dollars for larvicides, however effective, but if you do not dry up breeding pools, if you do not drain the country, if you do not conform to the principles of Ross, that there must be no stagnant water, you will have the mosquitoes, and as long as you have the Anopheles you will have malaria."

Thus the National Malaria Committee was originally oriented by its founder towards eradication. It is not evident, however, from a perusal of the papers presented at the Committee meetings during the next 25 years, that his objective was concurred in widely, much less enthusiastically, by the membership as a whole. The prevailing interest, judging by the publications, seemed to be in securing relief from a burden whose weight was so intolerable that the immediate objective of lightening it seemed much more important than the ultimate and somewhat visionary possibility of shedding it completely.

In 1941, the newly organized National Malaria *Society* listened to and pondered the significance of a paper by Drs. Fred Soper and Bruce Wilson (8). This was entitled "Species Eradication. A Practical Goal of Species Reduction in the Control of Mosquito-borne Disease." These gentlemen defined *species eradication* —a term which, in their opinion had been wrongly used to signify any degree of mosquito reduction—as "the complete extermination of the species under consideration in all phases of its development: ovum, larva, pupa, and imago." They went on to describe the techniques of eradicating *Aedes aegypti* and *Anopheles gambiae*. They related the evidence that the former species had been entirely eliminated from numerous large areas in Brazil; the latter, from all the infested portions of that country. With the vectors, went the diseases they had carried.[1] This was proof positive not only of the possibility but, in the case of these particular host-parasite-vector systems, of the practicality of large-scale area annihilation of insect-borne disease by destroying the essential vector.

This presentation must have made a deep impression on certain of the Society members because in 1943 two papers (9, 10) advocating malaria eradication objectives for this Nation were read at the 26th Annual Meeting in Cincinnati, Ohio. Dr. Wilbur Sawyer expressed his conviction that malaria in the United

[1] In the case of *A. gambiae*, the disease eradicated was *A. gambiae*-borne malaria. Other less important local vectors of malaria with different ecologic requirements were not eliminated by the anti-*gambiae* measures, so that malaria still persists in these areas.

States might be vanquished by exterminating the transmitters—a revival of the Hoffman credo—to be accomplished, according to Dr. Sawyer's plan, by application of the techniques of Soper and Wilson in malarious areas. The proposal of Dr. L. L. Williams, Jr., presented at the same meeting by Dr. Joseph Mountin (10), was also aimed at malaria eradication by wiping out focal endemicity. This was to be achieved by concentrating all classical antimalarial measures (DDT was not then available) in the areas of perennial transmission remaining in this country. Thus it is evident that a significant change in outlook had developed. American malariologists were no longer content to accept "control" as a sufficient objective of malaria reduction; the target from this point on was eradication.

This philosophy promptly manifested itself, as far as the National Malaria Society was concerned, in the following resolution (11) adopted at the Cincinnati meeting:

"Whereas: The malaria of the United States is being perpetuated in a limited number of endemic foci and

"Whereas: It is from these foci that malaria may epidemically extend over large portions of the country and

"Whereas: The return of soldiers with chronic infections may make future control more difficult and costly

"Therefore, be it resolved that the National Malaria Society endorse the program proposed by Dr. Joseph Mountin of the United States Public Health Service for the final eradication of malaria from the continental United States."

A copy was sent to the Surgeon General of the U. S. Public Health Service and, I have no doubt, made its appearance later at conferences with the Bureau of the Budget and Congressional Appropriations Committees. Due to a variety of circumstances, including the acquisition of experience and understanding regarding DDT, and the customary practice of planning federal budgets two years in advance, the five-year National Malaria Eradication Program of State and federal health services was not authorized until fiscal 1948 (July 1, 1947) (12). However, it is certain that the expression of confidence by the National Malaria Society in the feasibility and desirability of such an undertaking was a powerful influence in securing the necessary appropriations for this purpose.

From 1948 on, the Society was kept informed of the progress of the eradication efforts by a series of epidemiologic, entomologic, and operational papers (5, 12–20) presented at its regular meetings. As the program went forward, it became evident that serious consideration would have to be given in the near future to the determination of an acceptable endpoint at which eradication operations might be concluded. It was desirable that this be established by a neutral, highly respected group of malariologists. Accordingly, at the 1949 meeting in Memphis, I requested (17) that the National Malaria Society accept the responsibility of formulating criteria of malaria eradication which could be used in this or any other part of the world to indicate the approximate date at which endemic malaria might be said to have vanished from a particular area. The Board of Directors of the Society kindly recommended that the President

appoint such a committee, and this suggestion was ratified by the Society with the proviso that the final Committee report be circularized to Society members for comment.

During the following year, this *ad hoc* Committee,[4] under the chairmanship of Dr. E. H. Hinman, held two meetings at which many opinions and views were presented and analyzed. As the result of these deliberations, it was finally decided that "Malaria may be assumed to be no longer endemic in any given area when no primary indigenous case has occurred there for three years." (21). This conclusion, supplemented by appropriate definition of terms, conditions of reporting and investigation, and recommendations regarding the establishment of a national depository for malaria slides, was presented as a Final Report to the Board of Directors. It was made available to the members attending the Savannah meeting and was formally adopted by the Society on November 8, 1950. Thus the National Malaria Society officially sanctioned an arbitrary but carefully considered decision which, until it is improved upon, will be used to umpire the disappearance of endemic malaria from various parts of this country. It is believed that it will be of similar far reaching importance in gauging malaria eradication in other nations of the world.

As a final manifest of its interest in the general proposition of emancipating man from the bondage of paludism, the Society sponsored at its most recent meeting a symposium on "Nation-wide Malaria Eradication Projects in the Americas." This was arranged under the able direction of its then President, Dr. Paul F. Russell, and consisted of four summaries of national malaria reductive programs aimed at ultimate eradication of the endemic disease in the United States of America (12), in Venezuela (22), in British Guiana (23), and in Brazil (24), plus a general discussion of eradication principles (25).

The foregoing account indicates the manner in which the membership of the National Malaria Committee and Society promoted and assisted in the National Malaria Eradication Program of this country. As of this writing, the National Malaria Society criterion of malaria eradication has been fulfilled, as far as can be determined in 5 States, Alabama, Florida, Georgia, Kentucky, and Oklahoma; and it seems likely that this status will be achieved by the remainder of the traditionally malarious Southeastern States in the next few years. Thus malaria endemicity in the country as a whole is nearing the vanishing point. Because it appeared that the objective of the Committee was about to be reached, the justification for the future existence of the Society was questionable. Accordingly, tentative arrangements have been made for the National Malaria Society to consolidate with the American Society of Tropical Medicine to continue their joint existence as an organization in which the interests and activities of both groups would be blended and perpetuated. How then, will the present member-

[4] Dr. E. H. Hinman, Chairman; Dr. Charles N. Leach, Dr. E. F. Knipling, Mr. David Lee, Dr. E. C. Faust, Mr. Ralph S. Howard, Members; Dr. T. H. G. Aitken and Dr. Arnoldo Gabaldon, Corresponding Members; Professor John M. Henderson, Dr. F. C. Bishopp, Dr. Mark F. Boyd, Dr. Fred L. Soper, Dr. Thomas F. Sellers, Consultants; and Dr. Paul F. Russell, Dr. Justin M. Andrews, and Dr. Martin D. Young, *ex officio* Members.

ship of the National Malaria Society continue under its new aegis to press the cause for maintaining freedom from malaria in this country, and for bringing it about in other nations of the world?

The first and most important thing to do is to assist in extending eradication status to all the States in the Nation; the second is to help them maintain that status. As far as the membership of the National Malaria Society is concerned, I believe it should consider carefully the Malaria Surveillance and Prevention Program being operated jointly by the Public Health Service and the health departments of the 13 States which participated in the malaria eradication project. The program provides facilities for the epidemiologic investigation and appraisal of all cases of malaria reported or discovered by other means, and, if indicated by entomologic considerations, for the residual DDT spraying of all homes within a mile of proved cases. If the Society feels that this appears to be the most effective means of preventing transmission from relapsing or imported cases, its membership should support the program both individually and as a group. If some other more promising approach to the problem is available, it should be suggested to the responsible health authorities.

How long these mop-up activities should continue is a matter of conjecture at present. In the paper (12) which I read before this group last year, I suggested that surveillance and prevention should go on within each eradication State until the National Malaria Society criterion for the cessation of malaria endemicity was fulfilled—and that as each State met this requirement, it should carry on the program thereafter without federal assistance, unless malaria again became endemic and threatened to spread to other States. I believe that such a policy is sound in time of peace, but during wars, "police actions", or other mass enterprises abroad which result in the importation into this country of malaria-infected personnel on some considerable scale, the continuance of federal assistance for malaria surveillance and prevention is justified because of the federal creation of the problem, because of the federal-state-local investment in malaria eradication, and because, in some instances at least, the resulting load of case investigation and preventive operations is beyond the State's resources. This year, for example, something in excess of 6,000[5] infected service personnel have returned to this country from Korea (26). Efforts to neutralize their infectivity for mosquitoes are being made, but thus far more than half of them have been reported through civilian health channels from all but two States of the country. Their concentration in certain southern States is notable. Many of them are no longer under military control, and presumably any of these individuals may harbor gametocytes in their blood at unpredictable times until they are cured. The possibility of transmission from such sources can be minimized by apprehending them and by preventive DDT spraying. Similar impor-

[5] Information received from the Army states that from July 1, 1950 to August 31, 1951, 100,168 service personnel were returned from the Far East. During this period Army facilities reported 6,642 cases of malaria in Army personnel. It is not now known which of these cases resulted from exposure in Korea, but it is believed that very few, if any, were acquired in the United States.

tations on a larger or smaller scale may be expected from Korea or perhaps from other malarious theaters as long as the ideologic struggle continues. It seems to me that the malaria surveillance-and-prevention activities should be supported federally as long as the military introduction of cases continues to be a burden and a threat to States.

Another very important single source of malaria importation is the Mexican laborers who surge into the country each year, officially and surreptitiously. Their numbers are said to be on the increase and though indeterminate are on the order of several hundred thousand annually. Many of them come from highly malarious sections of Mexico, and malaria cases and deaths among them have, in fact, already been reported (27). Until recently, Mexicans have been employed principally in the western half of the country. This last summer, however, they were picking cotton in Georgia and neighboring States. This source of infection must be carefully watched by State and local health personnel and action taken, when indicated, to prevent the reestablishment of malaria in the U. S. A. It constitutes another compelling reason for continuing the cooperative Malaria Surveillance and Prevention Program until some better method of achieving and preserving eradication status can be devised.

The drastic reduction in malaria prevalence in this country has led many—including our distinguished representatives in Washington—to believe that no reason exists for us to continue a national or an individual interest in the disease. By some it is held that in exterminating malaria, the malariologist (assuming that he had something to do with the process) committed a species of professional hara-kiri. In my opinion, nothing could be further from the truth, and this brings me to the second phase of my story, namely, the ways and means by which members of the National Malaria Society can continue to influence malaria eradication objectives throughout the world. Briefly stated, these include energetic and persistent support of malaria research, a continuation of training in malariology and malaria control activities at home, and active participation in malaria prevention abroad.

The theory that enough is known about malaria and its prevention to discontinue expenditures for investigations on these subjects is a specious one. Great advances in knowledge about chemotherapy have been made in recent years—but the ideal antimalarial drug for treatment or for prevention is still to be discovered. Perhaps this depends upon knowing more about the life history of the parasite, the tissues it inhabits during incubation and latency, and the drug vulnerability of these cryptic stages. The weakest link in the plasmodial chain appears to be the gametocytes. We are still singularly uninformed about the factors or circumstances which occasion their appearance, or which determine their number, their sex, and their longevity. Gametocyteless strains of Plasmodium have been observed (28, 29). Can this condition be produced by man? Is there a substance which will kill or remove from the peripheral circulation either male or female gametocytes? Nothing more would be necessary to put an end to malaria transmission. Modern malaria control is

almost pathetically dependent upon DDT, a chemical which has served us well in combatting insect-borne disease. Yet against many vectoral species, it manifests the boomerang quality of generating a resistance to its own lethal effectiveness. When is this going to happen among the anophelines—and what are we going to do about it when it does? Only research, basic and applied, can offer reasonable prospects of success in solving these and related problems. In the interest of achieving malaria eradication in a shorter time and at less cost than is now possible, the members of our professional group should miss no opportunity to engage in, promote, or encourage support for research on malaria and its control in our laboratories, schools, institutes, foundations, and in the field.

The long-time approach to precluding the establishment of malaria endemicity in this country is, of course, its extermination in the other countries from which we derive many of our immigrants, e.g., Mexico, or, to put the matter in the more comprehensive language of the World Health Organization, . . ."the eradication of malaria from the world" (30). This sounds like a formidable task and, indeed, it is. But virtual elimination of the disease has now occurred in several areas, some of them vary considerably in size, and efforts to combat it in other countries are progressing on a scale hitherto undreamed of. American malariologists have played and are playing conspicuous roles in foreign malaria control activities. The pioneers in this undertaking were the agents of the International Health Division[6] of the Rockefeller Foundation, and such commercial enterprises as the United Fruit Company. Their current homologues are now operating under the aegis of these and other organizations such as the World Health Organization, the Pan American Sanitary Bureau, the Point 4 Program of the State Department, the Economic Cooperation Administration, the Institute of Inter-American Affairs, and their numerous collaborating agencies.

More malaria control specialists are needed abroad. One of our more eminent members, the architect of our National Malaria Eradication Program, spoke at length on this subject last year in Savannah (31). Dr. L. L. Williams, Jr., is now applying himself with his boundless enthusiasm to the task of mobilizing, coordinating, and integrating American resources for malaria control and other health services in distant lands. Thus if any of our members feel that the slump in the domestic market for malariologists precludes employment for them at home, I am sure that attractive positions abroad can be found without delay. They will have the great satisfaction of working to bring about freedom from malaria in another country, and thus of helping to secure the eradication status of our own.

Actually the number of Americans qualified and available to measure malaria prevalence and to direct malaria control programs overseas is not great enough to supply Dr. Williams' needs plus those of the Armed Forces, the latter of which will probably continue for some time. This means the production of more malariologists both at home and abroad. This nation possesses wonderful

[6] Now known as the Division of Medicine and Public Health.

malariologic competences in the classrooms, laboratories, and in the field. Those should be used to instruct young physicians, engineers, and biologists in their respective and collaborative phases of preventing malaria and other arthropod-borne diseases. These competences should be made available not only to American students but to selected foreign trainees professionally qualified and picked for leadership and teaching ability. These graduates, both American and foreign, should be used to direct control and eradication programs and to establish training centers overseas, especially for the necessary supervisors, foremen, and skilled laborers. The adequate instruction under prevailing local conditions of the men who must do the work is no less important than the production of key personnel in this or other countries where exceptional training facilities exist. Thus the membership of this Society by assisting in the development of competent corps of malaria control personnel in the other countries of the world increase the prospects of maintaining malaria eradication at home.

My account of the influence of this Committee and Society on malaria eradication in this country is probably incomplete. However, I believe the evidence shows that this multi-professional group, knit together by a common interest in malaria and a conviction that it could be eliminated from the U. S. A., has been, through its meetings, its publications, its resolutions, and its pronouncements, a powerful stimulus towards the accomplishment of this objective. Much progress has been made, the goal has been so near that three years ago serious consideration was given to enlarging the scope of the Society to include arthropod-borne diseases other than malaria. A committee was appointed to consider this possibility along with that of fusion with the American Society of Tropical Medicine or another closely related group, or of dissolution in the event that malaria eradication in the United States became an established fact (32). The Committee's recommendations were considered carefully by the Directors of the Society who voted last year in favor of the principle of amalgamating with the American Society of Tropical Medicine. Their action to form a new, larger, and more powerful association to be composed of individuals "interested in any phase of tropical medicine and tropical public health, including all arthropod-borne diseases" (33) has since been overwhelmingly endorsed by the members. Its professional areas of interest will include, according to the proposed constitution, "medicine, nursing, engineering, entomology, parasitology, and allied specialties."

This coalition may be effected at this meeting. If so, the name of our Society, for which many of us have a fondly sentimental attachment, will become history. I can see no reason, however, why its aims and objectives, and the future influence of its membership on malaria eradication should not be as much a part of the new society as they were the substance of the old. I trust that all the members of the National Malaria Society—and of the American Society of Tropical Medicine as well—will retain their connection with the new group and work unstintingly for it. If they do, it cannot fail to have as illustrious a career as its forebears.

Societas, ave atque vale!

REFERENCES

In future issues of this Journal, references will be cited by date and not by number.

1. Hoffman, Frederick L., A plea for a National Committee on the Eradication of Malaria, *South. M. J.* **9:** 413–420, 1916.
2. Hoffman, Frederick L., A plea and a plan for the eradication of malaria, *South. M. J.* **10:** 309–311, 1917.
3. Carter, Henry Rose, The malaria problem of the south, *Pub. Health Rep.* **34:** 1927–1935, 1919.
4. Williams, L. L., Jr., Economic importance of malaria control, *Proc. N. J. Mosq. Exterm. Assoc.* **25:** 148–152, 1938.
5. Welch, Sarah F., and Quinby, Griffith E., Epidemiologic appraisal of malaria in the United States during 1951. To be published.
6. Andrews, Justin M., Quinby, Griffith E. and Langmuir, Alexander D., Malaria eradication in the United States, *Am. J. Pub. Health* **40:** 1405–1411, 1950.
7. Hoffman, Frederick L., Progress and results in malaria eradication. Read before N. J. Mosq. Exterm. Assoc., Jan. 1918. Malaria Problems. The Prudential Insurance Co., Newark, 1928.
8. Soper, F. L., and Wilson, D. B., Species eradication; a practical goal of species reduction in the control of mosquito-borne disease, *J. Nat. Mal. Soc.* **1:** 5–24, 1942.
9. Sawyer, Wilbur A., A proposed program to prevent the spread of malaria in the United States from infected individuals returned from abroad, *J. Nat. Mal. Soc.* **3:** 61–67, 1944.
10. Mountin, J. W., A program for the eradication of malaria from continental United States, *J. Nat. Mal. Soc.* **3:** 69–73, 1944.
11. Minutes—1943 Meeting, *J. Nat. Malaria Soc.* **3:** 151–153, 1944.
12. Andrews, Justin M., Nation-wide malaria eradication projects in the Americas. I. The eradication program in the U. S. A., *J. Nat. Malaria Soc.* **10:** 99–123, 1951.
13. Hollis, Mark D., Malaria—its denouement?, *J. Nat. Malaria Soc.* **7:** 1–3, 1948.
14. Andrews, Justin M. and Gilbertson, Wesley E., Blueprint for malaria eradication in the United States, *J. Nat. Malaria Soc.* **5:** 167–170, 1948.
15. Tetzlaff, Frank, Malaria control progress and problems, *J. Nat. Malaria Soc.* **8:** 115–120, 1949.
16. Bradley, George H. and Goodwin, Melvin H., Jr., Malaria observation stations of the Public Health Service, *J. Nat. Mal. Soc.* **8:** 181–191, 1949.
17. Andrews, Justin M. and Gilbertson, Wesley E., Final phases of malaria eradication in the United States, *J. Nat. Malaria Soc.* **9:** 5–9, 1950.
18. Bradley, George H. and Lyman, F. Earle, Discussion of five years' use of DDT residuals against *Anopheles quadrimaculatus*, *J. Nat. Malaria Soc.* **9:** 113–118, 1950.
19. Quinby, Griffith E., Progress report of epidemiological appraisal of reported cases of malaria in the United States from 1947 through 1949, *J. Nat. Malaria Soc.* **9:** 397–400, 1950.
20. Quinby, Griffith E. and Welch, Sara F., Progress report of epidemiologic appraisal of reported malaria in the United States during 1949 and 1950, (in press).
21. Hinman, E. Harold, Chairman, Criteria of malaria eradication, Committee Report, *J. Nat. Malaria Soc.* **10:** 195–196, 1951.
22. Gabaldon, Arnoldo, Progress of the malaria campaign in Venezuela, *J. Nat. Malaria Soc.* **10:** 124–141, 1951.
23. Giglioli, George, Eradication of *Anopheles darlingi* from the inhabited areas of British Guiana by DDT residual spraying, *J. Nat. Malaria Soc.* **10:** 142–161, 1951.
24. Pinotti, Mario, The nation-wide malaria eradication program in Brazil, *J. Nat. Malaria Soc.* **10:** 162–182, 1951.
25. Soper, Fred L., General principles of the eradication programs in the Western Hemisphere, *J. Nat. Malaria Soc.* **10:** 183–194, 1951.

26. Dept. of the Army, Office of the Surgeon General. Letter, File No. MEDCE 710 (Malaria) dated November 7, 1951.
27. Fed. Sec. Agency, Pub. Health Serv., Nat. Office of Vital Statistics. *Weekly Morbidity Report* **2**(45): 1, 1951.
28. Huff, Clay G. and Gambrell, Elizabeth, Strains of *Plasmodium cathemerium* with and without gametocytes, *Am. J. Hyg.* **19:** 404–415, 1934.
29. Jeffery, Geoffrey M., Observation on a gametocyteless strain of *Plasmodium falciparum*, Presented at 1950 meeting of Nat. Malaria Soc.
30. Programme and Budget Estimates. Official Records of the World Health Organization, No. 18, p. 60. Geneva, 1949.
31. Williams, L. L., Jr., and Brady, F. J., Our role in international health, *Trop. Med. News* **8:** 17–20, 1951.
32. Hinman, E. Harold, The National Malaria Society: past, present, and future, *J. Nat. Malaria Soc.* **8:** 1–13, 1949.
33. Proposed Constitution and By-Laws of the American Society of Tropical Medicine and Public Health, 1951.

THE PRESENT STATUS OF MALARIA IN THE WORLD

PAUL F. RUSSELL

Division of Medicine and Public Health, The Rockefeller Foundation

INTRODUCTION

Malaria in recent years has been reported as far north as the Dvina River near Archangel in the USSR (65°) and as far south as Córdoba in Argentina (32°). Intense endemicity is still found in some areas of the Americas between 15°N. and 15°S., in Europe south of 45°N., in Asia south of 30°N., in Indonesia and the Philippines, Southwest Pacific (west of 180° and north of 20°S.) and Africa, north of 25°N. In the central and south Pacific, such islands as the Galapagos, Marquesas, Fiji, New Caledonia, New Zealand, Marshalls and Carolines, are entirely without anopheline mosquitoes and consequently are nonmalarious. During World War II *A. subpictus indefinitus* appeared on Guam but so far no indigenous malaria has been reported.

Transmission occurs as high as 2,591 meters (8,500 feet) near Londiani in Kenya, Africa, where *A. gambiae* and *A. funestus* shelter in native huts; at 2,770 meters (9,086 feet) in the Cochabamba region of Bolivia where *A. pseudopunctipennis* is the vector; and at 2,850 meters (9,348 feet) in Tadzhik SSR (Tajikistan) in south Central Asia where *superpictus* carries. The disease is also seen as low as the Dead Sea at 400 meters (1,312 feet) below sea level. Of course, between these limits of latitude and altitude malaria is a focal disease, not evenly distributed. In some countries, malaria is still an overwhelming handicap which makes economic and social progress impossible, in other lands malaria is a nagging nuisance doing steady damage in an unspectacular way, while elsewhere the disease is absent and has only indirect effects.

The advent of such insecticides as DDT marks a new stage in the long history of malaria because the residual sprays have demonstrated the economic feasibility of nation-wide malaria eradication. Already the epidemiology of malaria in some countries has been greatly modified and there is evidence that wide areas will be cleared, first of severe malaria and finally of the disease itself (1). But, in spite of outstanding successes this goal is farther ahead than many realize. The important local victories have not yet greatly reduced total world incidence.

Exactly how much malaria occurs annually is unknown. Only a small percentage of cases is reported officially and published reports are generally misleading. Some years ago I ventured an estimate of 300 million cases annually, with three million deaths (2). Recently, using population tables in the World Health Organization Demographic Yearbook (3) I attempted another estimate, based on personal and reading acquaintance with malarious areas. It seems to me probable that among the world's estimated total population of 2,378 millions there are occurring some 350 million cases of malarial fevers annually, with a mortality rate that very likely averages about 1 per cent. This is a guess. But I believe it gives a fair idea of the magnitude of the task involved in the elimina-

tion of malaria as a world-wide public health problem (4). Those who lately have been discounting malaria as no longer of much importance are invited to check into this estimate and, if it is sustained, to revise their thinking. Malaria today is probably the most prevalent serious illness of man.

As a basis for discussing the distribution of endemic malaria in more detail, the best definition of endemicity seems to be one formulated by the National Malaria Society (5). Malaria may be said to be no longer endemic in any given area when no primary indigenous case has occurred there for three years. Of course, case finding and reporting should be adequate to reveal the facts.

MALARIA IN NORTH AMERICA

Malaria is not endemic in Canada and soon will probably cease to be so in the United States. But it remains a problem in Central America and the West Indies. The disease has been declining in the States since about 1875, prior to which time it had been a major affliction in many areas, even making an annual appearance as far north as Massachusetts (6). The retreat was slow and malaria remained important up to the period of World War I. In 1912–1915 the U. S. Public Health Service, on the basis of a survey of 12 southern states, estimated a million cases of malaria annually among a population group of 25 million, with incidence rates as high as 40.9 per cent in parts of the Mississippi delta.

The recession was accelerated after the first World War although the disease remained highly endemic in certain areas, with parasite rates up to 50 per cent in the children of a few rural communities as recently as the thirties. During the latter period there was also evidence of the power of malaria to reinvade areas from which it had apparently altogether receded. But in 1948–1950 the Public Health Service studied all cases reported in 13 southern states (7, 8). It revealed total reported cases as follows: 1948, 9,317; 1949, 4,012; and 1950 (through September) 1,819. Of these, 770, 381 and 586, respectively, were carefully appraised and only 242, 65, and 26, respectively, were found positive. The number of primary cases in 1949 appeared to be 19 and in 1950, only 7. Such data leave no doubt that malaria has nearly disappeared as an endemic disease in the United States.

In the fight against malaria, Central America and the West Indies have not had the influence of climate on their side to the same extent as has the United States, although in some areas prolonged annual dry seasons have been limiting factors, of which full advantage has not yet been taken. Wherever there are prolonged cold or dry periods the effect of all control factors becomes greater and it seems easier to dislodge malaria.

In Tobago, with cooperation of The Rockefeller Foundation, the Trinidad government is attempting complete eradication of malaria by use of larvicides and residual DDT (12). Elsewhere in the West Indies and Central America a good deal of DDT is being applied. For instance, Costa Rica, El Salvador, Guatemala, Honduras, British Honduras and Nicaragua joined in a simultaneous campaign, chiefly oriented for malaria control, during 1950–51, using DDT residual spraying against household insects (13). Malaria is beginning to

retreat in all of these countries. In Panama, which had an earlier start, malaria is no longer a common disease. In Mexico the antimalaria campaign is gaining momentum but the disease is still a major problem.

RECESSION IN UNITED STATES

Speculation as to why malaria has retreated from the United States is interesting. So far as one can determine there have been no significant changes in the invasive properties of the parasite, the vectorial powers of the insect, or the immune defenses of the population (9). Americans overseas readily become infected; paretics and volunteers at home can be easily inoculated with local strains of plasmodia by the local anopheline vector; and the latter will readily transmit exotic strains.

Presumably there must have been an over-all reduction in the numbers of the vector, *A. quadrimaculatus*, since 1875. This would naturally follow as a result of the vast amount of land improvement for purposes of agriculture and real estate, as well as the extensive planned antimosquito drainage since 1915. It seems unreasonable to doubt that *vector reduction* has been one factor in the decline of malaria even though there are many areas formerly malarious but now completely free of the disease, where the density of the vector anopheline is probably as high or even higher than ever (10). No quantitative collection records exist whereby such assumptions can be checked, but they seem reasonable.

There were antimalaria drainage projects at the turn of the century in a number of places as widely separated as Long Island and Sierra Leone, Brazil and Hong Kong. The most notable were in Malaya directed by Watson, and in Cuba and the Panama Canal Zone by Gorgas, Le Prince and associates. The latter, in particular, had wide publicity but the vastness of the Canal project caused many in the United States to believe that malaria control by antimosquito measures was too costly for an average community. Therefore not much deliberate antimalaria drainage was done until after 1916 when The Rockefeller Foundation, in cooperation with the Department of Health in Mississippi and the Public Health Service in Arkansas, set up experiments to determine whether or not malaria could be controlled at "costs which the average community could afford." These experiments and similar studies soon made it clear that malaria control was economically feasible and they marked the beginning of a very extensive attack on rural malaria.

During World War I the U. S. Army in cantonment areas and the U. S. Public Health Service outside the military camps carried out no fewer than 43 antimosquito projects in 15 states, protecting one and three quarter million civilians and over 800,000 military personnel. Then, from 1933 to 1935, it is estimated that the Works Progress Administration, and its predecessor agency, constructed 32,000 miles of average-size ditches, draining 623,000 acres of land, a definite antimalaria factor in spite of obvious and great defects in conception and operation of this huge project (9). During the second World War the Army, again in its cantonments and the Health Service in extra-cantonment areas,

cooperating with state health departments, carried out no fewer than 2,200 mosquito control projects in 19 different states. This must have had a supressing effect on malaria. It certainly protected the areas involved almost completely. Finally, one should note the Tennessee Valley program and the well-directed routine state and county mosquito control projects which have involved the expenditure of large sums for antimalaria measures.

Probably many other factors have taken part in the recession of malaria (11). For example, one recognizes the economic advance of the South. This has permitted better housing and education, greater use of doctors, hospitals, therapeutic and prophylactic drugs, insecticides (specially household pyrethrum sprays), drainage, river training, screening and other antimosquito measures. The resulting increase in cattle population was followed by a greater shift of vector from man to animal. Also, there has been an acceleration of urbanization in the South. Finally, tremendous numbers of gametocyte carriers have moved from rural malarious areas of the South into nonmalarious northern communities.

One enjoys speculating on cause and effect in these changes. Prior to the twenties, hookworm disease and malaria, two anemia-producing, inertia-inducing illnesses, seemed to hold the South in servitude. Very great strides toward the eradication of these maladies were made in the twenties and thirties. Prompted possibly by the improvement in public health, there was simultaneously a striking advance in the South in road building, schooling, agricultural practices, animal husbandry and industrialization. All, no doubt, interacted for the common good.

The fact that malaria incidence in the United States began to decline before there were antimalaria projects based on a knowledge of transmission should not detract from the importance of conscious measures of control during the 20th century. Nor should such factors as the reduction in the price of quinine from about $4.50 per ounce in the 1880's to 25 cents in 1913 be overlooked. There has been no single magic factor but rather many interlocking ones, the more influential of which came into play slowly after the discoveries of Ross, Bignami, Bastianelli and Grassi in 1897–1898. Just because there had been a gradual recession prior to the intensification of planned control about 1915, one should not underestimate the effect of conscious efforts at malaria control since then. Nor should one assume that it is today a waste of funds to continue some planned malaria control measures, particularly those which accomplish environmental changes. Experience thus far in areas formerly heavily infested gives no adequate basis for such an assumption. Periodic suspension of the use of insecticides for one or two years at a time should, however, have careful experimental testing on an ever-widening basis. Perhaps the cost of malaria control could thus safely be reduced in areas from which the disease has apparently disappeared. Such interruption in the use of organic toxicants might also help to avoid development of insect resistance, common in houseflies and expected some day in anophelines.

MALARIA IN SOUTH AMERICA

Malaria in Argentina is sharply limited to the northern sections. It is spread by *A. pseudopunctipennis* in the northwest and by *A. darlingi* and *A. albitarsis* in the northeast. An eradication project with residual DDT has already so reduced the incidence of the disease that it is definitely approaching an end point. In Bolivia, some 70 per cent of the territory is still malarious (14) but the use of DDT is beginning to make itself felt in a few areas. Brazil has been highly malarious; at least half of its population requires protection. But the epidemiology of the disease is rapidly changing because of a nation-wide program of DDT house spraying, in progress since 1947 (15). About 8,000 workers are engaged in this large project which includes 80 per cent of all houses in malarious areas in Brazil. The 1950 budget, from various sources, was equal to nine and a half million dollars. Over two and a half million houses were sprayed. Malaria is falling back under such a tremendous assault and will become much less important in Brazil in the relatively near future.

Chile reported the eradication of its only malaria vector, *A. pseudopunctipennis*, in 1948 (13). Uruguay has no endemic malaria. British Guiana has been highly malarious but the disease has now been practically eliminated from the coastal plain by DDT residual spraying (16, 17). In French Guiana, DDT has been very successful (18). In Ecuador, Peru and Colombia DDT is also beginning to modify the incidence and distribution of malaria. Probably the greatest change has occurred in Venezuela (19) where active DDT spraying has been carried on since the end of 1945 with increasing intensity. Today over 85 per cent of all houses in the malarious areas are sprayed. The malaria death rate had fallen from a median of 112, in 1941–1945, to 9 in 1949. Malaria is becoming relatively unimportant as a public health problem. The Venezuela project has special significance for it was the first nation-wide DDT scheme, the first planned from the outset to eliminate malaria from an entire country by residual spraying.

All in all, perhaps the most important fact that emerges from this brief survey of the status of malaria in the Americas is that we now have an economical method which can be applied by a nearly standardized technique throughout the malarious parts of the Western Hemisphere.

MALARIA IN EUROPE

Malaria is no longer a common disease in Italy, Greece or Cyprus, where nation-wide malaria eradication projects have been in operation. Good control is also in effect in Corsica (20, 21), Portugal and Yugoslavia (14). The change in these countries since the late thirties and the years of World War II has been dramatic. For example, in Italy malaria morbidity per 100,000 population was 974.0 in 1905, 826.7 in 1917, 382.2 in 1935, 900.6 in 1945, and 7.5 in 1950. Residual spraying alone has reduced the density of the vectors *A. sacharovi* and *A. labranchiae* to a remarkably low incidence in such areas as the classical "home" of malaria in the Pontine Marshes. In Sardinia not a single primary case of malaria was proved in 1950 and there was but a single case in 1951.

The vector, *labranchiae*, is still present but has been sharply reduced in numbers (14, 20, 22, 23). In Greece, in 1905, it is said that there were a million cases of malaria in a population of two and a half millions. In 1951 malaria rates had fallen to the point where it was considered safe to stop DDT residual spraying in most of the Peloponnesus and Crete. Total malaria parasite indices in Greece are down to 0.1 per cent (14, 20, 24). The same applies to Cyprus where anophelines have almost been eradicated (20, 25).

In Yugoslavia, attendance at antimalaria centers was up to 244,769 in 1937 but down to 8,562 in 1949, in spite of the fact that there had been a considerable increase in malaria during World War II (14). Portugal and Spain are still malarious, the latter highly so in some areas (14, 20). Active DDT spraying has greatly reduced the incidence of malaria in Portugal where malaria is carried by the relatively zoophilic *A. atroparvus* (20). Spain has been seriously handicapped by shortages of material but is now expanding a residual spraying program.

Malaria persists lightly in the Netherlands (26) and in Germany (27–30). It increased considerably in Germany and to some extent in the Netherlands during the last war and the years following. Belgium is not malarious and did not become so in spite of war-flooding which resulted in increased anophelism (37).

Not much recent information is available about malaria incidence in countries behind the Iron Curtain. We know that there was a great deal of malaria during World War II and the post-war years in Hungary (31), Czechoslovakia (32), Poland (33), Rumania (34) and the USSR (35, 36). Just prior to the USSR's entry into the war, suppressive therapy with acriquine was provided to some 3.5 million persons in Russia (36). Undoubtedly there was a considerable increase in all of these countries after the war (37).

War Heightens Malaria Endemicity

War nearly always intensifies malaria of whatever endemicity and sometimes it transforms potential into actual endemicity. Many factors contribute and the fact that not infrequently the numbers of vector mosquitoes and human carriers are simultaneously increased in an area adds to the danger of malaria epidemics.

For example, there is often a breakdown in administration. Health department activities, malaria control services, distribution of antimalarials for treatment and prophylaxis, medical treatment in hospitals, dispensaries and homes are curtailed, disrupted or entirely stopped for lack of authority, funds, materials, personnel, transport, or because of local fighting or the fear of it.

Then there is the factor of disruption of the community. Normal facilities, including food and clothing supplies, are reduced or unavailable. People may be forced to live precariously in partially destroyed houses, or farm buildings, or in open fields, forests or caves through fear, actual warfare or war damage. There may be overpopulation of endemic areas, movement of nonimmunes into malarious areas or of gametocyte carriers into potentially endemic areas. The mass movement of civilians or troops, transfer of workmen, guerrilla settle-

ments, repatriation of displaced persons, return of war prisoners, demobilizing of malarious soldiers—all are factors which may contribute greatly to increase malaria incidence. The killing or removal of livestock and the destruction of stables may tend to force zoophilic species to feed more often on man. The stabling of military animals in the middle of a village may attract unusual numbers of a vector species.

Finally, there is the creation of breeding places for vector mosquitoes. There may be the opening up of jungle areas; diverting of streams; desertion of open mining works; damage to sewage, water pipes, dikes, irrigation and drainage systems; shell, bomb and mine craters; trenches; fox-holes; tank traps; vehicle ruts; increased number of stagnant pools; rubble from demolished banks, bridges and buildings; damming streams; drainage blocked by hastily built airfields, highways, causeways and assorted military installations. All these and still other factors may operate to increase malaria endemicity.

On the other hand, in some cases the effective measures taken by military authorities and the aid given by them to local communities may tend to reduce the incidence of malaria in communities. But throughout all history war has generally increased the incidence of malaria and this was true of World War II (27–30, 32–35, 37–41, 46).

Regression in Europe

Malaria was fairly widespread in Finland and Sweden in the 19th century (42). Malaria regression in Finland dates from about 1900 and in Sweden from about 1860, although there were a few cases in the latter country in 1939. So, too, malaria was much more prevalent in Germany prior to 1850 and had regressed fairly steadily from that time until World War II (43). Alsace was malarious up to about 1885 but the disease has since practically disappeared in France and did not re-occur during the war (44). England also had a good deal of malaria up to the middle of the last century but it has now almost disappeared (44, 46, 47). Interestingly, the distribution in 1860 appears to have been almost identical with that in 1929 (45). There has been much less malaria in England following the second world conflict than after the first (46).

The reasons for the regression of malaria in England and northern Europe are probably in part the same as those already discussed in explaining the retreat in the United States. No doubt the increasing availability and lower cost of quinine was one factor (42, 45, 46). Soil drainage and river training probably also had a strong influence (43, 44). The habits of the noncarrier *A. typicus* are such that it is adapted to cultivated lands. Its larvae are found in small, shallow breeding places, often artificial, with scanty vegetation and subject to major temperature changes. Hence, this zoophilic species remains probably more abundant than ever. But the vector *A. messeae*, in its aquatic stages, typically occurs in large, densely overgrown breeding places whose depths prevent extreme changes of temperature. Increased soil cultivation and river training have tended to eliminate such places and have thus very likely reduced the incidence of *messeae* (43). Finally, it is likely that progressive heightening of

social, economic, educational, medical and public health levels, with improved domestic hygiene, must also be taken into account.

However, in Europe as in the United States, there has no doubt been an interplay of many factors, not the least of which has been a lowering of the incidence of the vector and a lessening of its contact with man, in climates not ideal for malaria in the first place. Also, it seems likely that *messeae* has never been a really dangerous vector, for this species never seems to be so strongly attracted to man as are *atroparvus* and *labranchiae* (47). Some potentiality for malaria no doubt remains wherever the disease was once known. But only in such disturbances as war and major economic depressions can malaria now regain the ground it has lost. Modern technique and drugs for dealing with this disease sharply limit the dangers of any resurgence due to natural cyclic tendencies.

The story of malaria regression from Europe and the United States illustrates, among other things, the importance of environmental changes which lessen the incidence of vector breeding places and the degree of contact between the insect and man. In countries where malaria control is recent and consists entirely of residual spraying it would be wise not to overlook these nonchemical factors, but to carry out environmental sanitation and improvement of domestic hygiene to the fullest extent finances will permit. In addition to the obvious benefits such measures would bring, it seems likely that much money might be saved later by periodic curtailment of the use of insecticides, without fear that malaria would recur as a public health problem.

MALARIA IN ASIA

There is still much malaria in the Near East (45, 47). In Turkey, endemicity is highest in the coastal zone of some 25–50 miles width, both north on the Black Sea and south on the Mediterranean (48, 49). But an active DDT spraying project is being carried out effectively. In 1948, for example, some 70 tons of this insecticide were sprayed in some 10,000 villages to protect about two million people (46). Israel has been controlling malaria for a number of years (47). There have been demonstration malaria control teams of the World Health Organization in Lebanon, Iran and Afghanistan which have made a good start, but malaria is still abundant in these countries, as it is in Syria, Iraq and the other Arab kingdoms (4, 47).

In Pakistan WHO demonstration teams have reduced the degree of malaria endemicity in a few areas (4). But except in these and on certain large agricultural estates malaria continues to be a serious problem, as it is in India, Burma, Malaya, Thailand, Cambodia, Vietnam, Indochina, Indonesia, China and the Philippines (14, 20, 50–55). Korea has seasonal malaria, fairly severe in some areas. Japan is only lightly affected (50).

Ceylon in 1946 became the first country in Asia to start a nation-wide malaria control scheme with residual DDT. Previously, the disease had constituted its greatest public health problem. Now a dramatic change has occurred following effective control. The malaria death rates, which from 1940 to 1946 fluctuated

between 854 and 880 per 100,000 population, fell in 1947 to 663, in 1948 to 473, in 1949 to 329, and in 1950 to 215. Infant mortality, which was 177 in 1928, was only 79 in 1950 (14, 20, 56, 57).

Thailand is engaged in an active DDT spraying program and may reasonably be expected to reduce malaria greatly within the next few years (20). Malaya continues its excellent control in urban areas and on the larger rubber estates.

In India there has been a convincing demonstration of the effectiveness and economic feasibility of DDT spraying. A program was started in Bombay State in 1946 and has been continued ever since (14, 20, 58, 59). This project now embraces some two million habitations and nine million people; and its annual cost is less than 10 cents per person protected. During 1949 it was estimated that some 500,000 cases of malaria were prevented. Elsewhere in India there are WHO demonstration teams (4) and here and there one also sees excellent local control projects.

So far as can be determined, malaria remains at about the same level in the rest of the Far East, probably intensified by World War II and subsequent local disturbances.

MALARIA IN AUSTRALIA AND PACIFIC ISLANDS

Melanesia still has many areas of high endemicity and represents a great reservoir of malaria. The disease is a major health hazard in New Guinea, New Britain, New Ireland, the Admiralty, Solomons and the New Hebrides Islands. It is not a serious problem in Australia although there are still some foci north of 19°S. (20, 60–63).

MALARIA IN AFRICA

The status of malaria in North, Central, East and West Africa remains about the same as in the past. The disease varies in incidence from hypo- to holoendemicity in most areas not yet under serious attack. In Egypt some areas, including the large oases, have had DDT residual spraying since 1945 and are showing a lessened incidence. The potent vector *A. gambiae* threatened to intensify malaria in the Nile Valley in 1943–1944 but was successfully eradicated.

Extensive malaria control projects have been in progress for some years in the Union of South Africa and DDT has added to the effectiveness of this work. DDT has had successful trials in Southern Rhodesia and there is a large house-spraying program in Madagascar, involving so far a population of more than one million. In Mauritius, an *Anopheles* eradication project is being attempted and results to date have been excellent. But in general, Africa is still a highly malarious continent (14, 20, 47, 64–79).

CONCLUSIONS

Residual spraying with such toxicants as DDT has begun to change the status of malaria in the world. Already this new technique has almost eliminated malaria from many foci, including entire countries. Methods are fairly well standardized and can be applied on a nation-wide scale at financially feasible

costs almost anywhere. But malaria is still probably the most prevalent serious illness of man and it should continue to receive major attention and high priority from those faced with the problems of international disease control.

The greatest handicaps today in the control of malaria are lack of (a) trained personnel and (b) governmental antimalaria services within effective national health organizations.

Three major questions are currently being asked. First, will anopheline vectors become resistant to residual insecticides; second, is malaria control safe in holoendemic areas where the population has a strong tolerance to the disease; and third, is control worthwhile in areas which are or may be overpopulated?

Without presuming to answer them, one may comment on these questions briefly.

First, there is no clear record of practical anopheline resistance to an insecticide, but rumors are rife, from Greece and Sardinia to the Tennessee Valley (20). Since both *Culex* and *Aedes* species, not to mention *Musca*, have become resistant, it seems reasonable to expect trouble and to think about countermeasures.

Second, the need for control is probably much greater in holoendemic areas than appears on the surface. If one is planning routine malaria control there is no logical reason to omit tolerant populations (78). Obviously, if one merely "tampers" with the situation, with no plans for continuity of effort, there could be epidemics after the community had lost its tolerance. However, modern drugs and sprays are so effective that, without gross neglect, serious outbreaks are not likely.

Third, to this writer there appears to be no logical reason for slackening or stopping malaria control because of fears about increased populations pressure and concomitant evils. But, since our aim is a healthier world and not bigger populations, the need is apparent for an integration of malaria control activities with those of agriculturists, economists, educators, health officers and social scientists for the common good (80, 81).

REFERENCES

In future issues of this Journal, references will be cited by date and not by number.

1. Russell, P. F., Some epidemiological aspects of malaria control with reference to DDT, *Jl. Nat. Mal. Soc.* **10:** 257–265, 1951.
2. *idem*, Malaria and its influence on world health, *Bull. N. Y. Acad. Med.* **19:** 599–630, 1943.
3. Demographic Yearbook 1949–1950, Dept. Social Affairs and Dept. Economic Affairs, WHO, New York, 1950.
4. Russell, P. F., Malaria control activities of the World Health Organization, *Jl. Nat. Mal. Soc.* **9:** 1–4, 1950.
5. Criterion of cessation of malaria endemicity, *ibid.* **10:** 195–196, 1951.
6. Faust, E. C., Clinical and public health aspects of malaria in the United States from an historical perspective, *Am. Jl. Trop. Med.* **25:** 185–201, 1945.
7. Andrews, J. M., Quinby, G. E., and Langmuir, A. D., Malaria eradication in the United States, *Am. Jl. Pub. Health* **40:** 1405–1411, 1950.

8. Andrews, J. M., The eradication program in the U. S. A., *Jl. Nat. Mal. Soc.* **10:** 99–123, 1951.
9. *idem*, What's happening to malaria in the U. S. A., *Am. Jl. Pub. Health* **38:** 931–942, 1948.
10. Goodwin, M. H., Jr., Observations on the recession of malaria in an area of southwestern Georgia, *Am. Jl. Trop. Med.* **30:** 651–661, 1950.
11. Ackerknecht, E. H., Malaria in the upper Mississippi valley 1760–1900, *Suppl. Bull. Hist. Med.* No. 4, 142 pp., 1945.
12. Trinidad Govt., *Ann. Rep. Mal. Div. Health Dept.* Trinidad and Tobago, 89 pp. mimeo. 1949.
13. Soper, F. L., General principles of the eradication program in the western hemisphere, *Jl. Nat. Mal. Soc.* **10:** 183–194, 1951.
14. Pampana, E. J., Large-scale malaria control campaigns using residual insecticides, WHO/MAL/46. Mimeo. 59 pp., 25 Sept. 1950.
15. Pinotti, Mario, The nation-wide malaria eradication program in Brazil, *Jl. Nat. Mal. Soc.* **10:** 162–182, 1951.
16. Giglioli, G., Eradication of *Anopheles darlingi* from the inhabited areas of British Guiana by DDT residual spraying, *ibid.* **10:** 142–161, 1951.
17. van der Kuyp, E., Contribution to the study of malaria epidemiology in Surinam, *Trop. Dis. Bull.* **48:** 109–10, 1951.
18. Floch, H., Les résultats de la "dédétisation" en Guyane française: destruction de *Aedes aegypti* et réduction spectaculaire du paludisme, *Rev. Paludisme et Méd. Trop.* **9:** 49–57, 1951.
19. Gabaldon, A., Progress of the malaria campaign in Venezuela, *Jl. Nat. Mal. Soc.* **10:** 124–141, 1951. See also, The nation-wide campaign against malaria in Venezuela, *Trans. Roy. Soc. Trop. Med. & Hyg.* **43:** 113–160, 1949.
20. Personal communication.
21. Cavaillon, A., Adam, J. P., Bernard, L., Camarin, R., Jaujou, C. and Trinquier, E., Deux années de lutte contre le paludisme en Corse: premiers résultats, *Bull. Nat. Méd.*, Paris. **134:** 320–325, 1950.
22. Missiroli, A., The control of domestic insects in Italy, *Am. Jl. Trop. Med.* **30:** 773–83, 1950.
23. Logan, J. A., *A. labranchiae* eradication in Sardinia: an interim report, *ibid* **30:** 313–23, 1950.
24. Livadas, G. A., Kotoyannaki, F. K., and Issaris, P. C., Malaria control in Greece during the four year period 1946–1949, *Riv. di Malariologia.* **28:** 247–290, 1949.
25. Shelley, H. and Aziz, M., Anopheles eradication in Cyprus, *Brit. Med. Jl.*, Apr. 30, 767–768, 1949. See also Aziz, M., *Cyprus Ann. Med. and San. Rep.* pp. 13–16, 1949.
26. Swellengrebel, N. H., The malaria epidemic of 1943–1946 in the Province of N. Holland, *Trans. Roy. Soc. Trop. Med. and Hyg.* **43:** 445–464, 1950.
27. Hormann, H., Malaria in Deutschland 1945–1947, *Ztsch. f. Tropenmed. u. Parasit.* **1:** 32–91, 1949.
28. Schroeder, W., Malariaepidemien im östlichen Norddeutschland nach dem zweiten Weltkriege, *ibid.* **1:** 488–511, 1950.
29. Steiniger, F., Die Malaria Überträger Schleswig-Holsteins, *ibid.* **2:** 94–106, 1950.
30. Weyer, F., On the present malaria situation in Germany, *Trop. Dis. Bull.* **45:** 150–51, 1948.
31. Zoltai, N., Malaria in Hungary, *ibid.* **46:** 896–897, 1949.
32. Snuparek, J., Malaria in Czechoslovakia after World War II, *Jl. Parasitology* **33:** 506–508, 1947.
33. Bincer, W., Malariological problems in Poland, *Trop. Dis. Bull.* **46:** 999–1000, 1949.
34. Ciuca, M., The campaign against malaria in Rumania, *ibid.* **48:** 441–442, 1951.
35. Beklemischev, V. N., *ibid.* **44:** 639–640, 1947.

36. Moshkovski, S., Measures employed in the control of malaria in Soviet Russia, Boyd's Malariology, W. B. Saunders Co., Philadelphia, Vol. II, pp. 1424–1431, 1949.
37. Pampana, E. J., Malaria in Europe 1938–1947, *Epidem. and Vital Stat. Rep.* WHO Vol. I. No. 18. 392–409, 1948.
38. Canaperia, G. A., Malaria in Italy during the war and post-war period, *Bull. Off. Int. d'Hyg. Pub.* **38:** 1070–1076, 1946.
39. Neild, F. G. Mosquito control in the British sector of Berlin in summer 1949, *Jl. Roy. Army Med. Corps.* **95:** 222–229, 1950.
40. Möllhausen, W., Zur Virulenz der griechischen Malaria, *Ztsch. f. Tropenmed. u. Parasit.* **2:** 75–78, 1950.
41. Sautet, J., Etat actuel du paludisme et de l'anophélisme dans la région méditerranéenne, *Rec. Travaux. Inst. Nat. Hyg.*, Paris **2:** 176–196, 1944.
42. Ekblom, T., Studies on malaria and Anopheles in Sweden and Finland, *Trop. Dis. Bull.* **46:** 205–e, 1949.
43. Fessler, K., Fischer, L. and Sorg, G., Anophelesfunde in Südwürttemberg und ihre Beziehung zum Problem der einheimischen Malaria, *Ztsch. f. Tropenmed. u. Parasit.* **1:** 458–488, 1950.
44. Callot, J., Le paludisme en Alsace et en Moselle, *Ann. Parasit. Humaine et Comparée.* **23:** 289–295, 1948.
45. James, S. P., The disappearance of malaria from England, *Proc. Roy. Soc. Med.* **23:** 71–85, 1929.
46. Shute, P. G., A review of indigenous malaria in Great Britain after the War of 1939–1945, compared with the corresponding period after the 1914–1918 war, *Mon. Bull. Min. Health and Pub. Health Labor Ser.* **8:** 2–9, 1949.
47. Hackett, L. W., Conspectus of malaria incidence in northern Europe, the Mediterranean Regions and the Near East. Boyd's Malariology. W. B. Saunders Co., Philadelphia, Vol. II, pp. 788–799, 1949.
48. Okan, S., Malaria control in Turkey, *Trop. Dis. Bull.* **47:** 595, 1950.
49. Nlyan, A., Die geographische Verteilung der Malaria in der Türkei, *Ztsch. f. Tropenmed. u. Parasit.* **2:** 338–350, 1951.
50. Covell, G., Malaria incidence in the Far East, Boyd's Malariology, W. B. Saunders Co., Philadelphia, Vol. II, pp. 810–819, 1949.
51. Rao, V. V., A critical review of malaria control measures in India, *Ind. J. Malariology* **3:** 313–326, 1949.
52. Macdonald, G., Residual insecticides in Assam, *Brit. Med. Jl.*, Aug. 12, p. 414, 1950.
53. Farinaud, M. E., Choumara, R., Royer, J., Malaria infestation in Southern Indo-China, *Trop. Dis. Bull.* **48:** 6–7, 1951.
54. Dy, F. J., Malaria problem in the Philippines today, *J. Philippine Med. Assoc.* **25:** 347–350, 1949.
55. Malaria Advisory Board, Kuala Lumpur, Fed. of Malaya. Ann. Rep. 19 pp., 1949.
56. Rajendram, S., Administration report of the Superintendent Anti-Malaria Campaigns for year 1950. Mimeographed, 1951.
57. Rajendram, S., Cader, M. H. M. A., and Visvalingham, T., Malaria eradication in Ceylon, *Nature* **166:** 486, 1950.
58. Viswanathan, D. K., Malaria and its control in Bombay State, Connaught House, Poona, 263 pp., 1950.
59. Viswanathan, D. K., and Ral, T. R., Control of rural malaria with DDT-indoor residual spraying in Kanara and Dharwa Districts, Bombay State: Third year's results 1948–1949, *Ind. Jl. Malariology* **3:** 269–312, 1949.
60. Lambert, S. M., Malaria incidence in Australia and the South Pacific, Boyd's Malariology, W. B. Saunders Co., Phila., Vol. II, pp. 820–830, 1949.
61. Ford, E., The malaria problem in Australia and the Australian Pacific Territories, *Med. Jl. Australia* **23:** 749–60, 1950.

62. Black, R. H., Anophelism without malaria in Northern Australia, *Ann. Trop. Med. and Parasit.* **44:** 207–211, 1950.
63. Roberts, F. H. S., The distribution and seasonal prevalence of anopheline mosquitoes in North Queensland, *Proc. Roy. Soc. Queensland*, **59:** 93–100, 1948.
64. Wilson, D. B., Malaria incidence in Central and West Africa, Boyd's Malariology, W. B. Saunders Co., Phila., Vol. II, pp. 800–809, 1949.
65. Dana, R., Corcos, A., and Corcos, V., Gravité de la poussée épidémique actuelle de paludisme en Tunisie, *Bull. Soc. Path. Exot.* **43:** 416–418, 1950.
66. Hugonot, R., Une épidémie de paludisme au Sahara, *Arch. Inst. Pasteur d'Algérie* **28:** 469–508, 1950.
67. Olivier, L. J. G., Organized malaria control in Northern Transvaal, *Public Health* Johannesburg **14:** 375–379, 1950.
68. Bernard, P., La prophylaxie anti-palustre au moyen des insecticides de contact à Madagascar, *Bull. Soc. Path. Exot.* **43:** 502–512, 1950.
69. Dowling, M. A. C., Report on progress of malaria eradication scheme, Gov't Printer, Port Louis, Mauritius. 23 pp., 1949.
70. *idem* An experiment in the eradication of malaria in Mauritius, *ibid.* 11 pp., 1950 (See also WHO/Mal/61, 1950, and Bull. WHO, **4:** 443–461, 1951).
71. *idem* Malaria eradication scheme, Mauritius, *Nature*. Jan. 13, 67–68, 1951.
72. Bruce-Chwatt, L. J., Malaria in Nigeria, *Bull. W. H. O.* **4:** 301–327, 1951. See also Summary on malaria in Nigeria. 27 pp. mimeo. WHO/Mal/43, 8 Aug., 1950.
73. Annecke, S., Report on malaria control in Transvaal, 37 pp. mimeo. WHO/Mal/48, 3 Oct., 1950.
74. *idem* Economic importance of malaria. 6 pp. mimeo. WHO/Mal/57, 24 Oct., 1950.
75. Blair, D. M., Report on malaria control in Southern Rhodesia, 8 pp. mimeo. WHO/Mal/49, 3 Oct., 1950.
76. Cambournac, F. J. C., Report on malaria in equatorial Africa, 113 pp. plus annexes, mimeo. WHO/Mal/58, 26 Oct., 1950.
77. Macdonald, G., The economic importance of malaria in Africa, 17 pp. mimeo. WHO/Mal/60, 24 Oct., 1950.
78. Malaria Conference in Equatorial Africa, WHO. Tech. Rep. Series No. 38. 72 pp. April, 1951.
79. De Meillon, B., Malaria survey of South-West Africa, *Bull. WHO* **4:** 333–417, 1951.
80. Russell, P. F., Malaria and society, *Jl. Nat. Mal. Soc.* **10:** 1–7, 1951.
81. *idem* Public Health Practice and Population Pressure in the Tropics, *Am. Jl. Trop. Med. & Hyg.* in press, 1952.

THE TREATMENT OF MALARIA

L. T. COGGESHALL

Dean, Division of Biological Sciences, University of Chicago

Prior to the discovery of the sulfonamides and antibiotics, few diseases, if any, responded more favorably to a drug than did malaria to quinine. The importance of this drug to civilization can never be overestimated. Nevertheless, over the centuries the natural incidence of the disease was not altered by quinine or later by quinacrine (Atabrine) because of a common shortcoming, the inability to eradicate an infection completely. Their usefulness was largely limited to the suppression of the clinical manifestations of the infection or the early termination of the acute attack. However, since malaria is chiefly characterized by its tendency to relapse, particularly *vivax* infections which is the more prevalent variety, the constant search by scientists for improved compounds continued. These efforts were relatively meager and sporadic until the Germans began a systematic and intensive program following World War I. From this effort they were rewarded with the discovery of quinacrine, an important substitute for quinine, and pamaquine, potentially a curative but relatively toxic compound. Again there was a relative lull in the search for new drugs until it became apparent that an impending war would involve millions of susceptible individuals in some of the most malarious areas of the world.

In 1939 a committee was formed by the National Research Council to initiate an rapidly as possible an antimalarial chemotherapeutic research program. A very intensive effort, involving many of the country's most able investigators, gradually developed and ultimately resulted in the discovery of several improved compounds. Although the program diminished in volume after World War II, a few laboratories have maintained an active interest and have made major contributions to the chemotherapy of malaria. These new compounds have been sufficiently evaluated so that tentative estimates which regarded them as superior suppressives and curative compounds are now established.

RATIONALE FOR THE TREATMENT OF MALARIA

The greatest misconceptions about the treatment of malaria, especially in the past, have arisen from the fact that too many considered it a single disease. Malaria is not a disease—it is a variety of diseases.

After the diagnosis has been made from the symptoms and demonstration of the parasite involved, it is too commonly believed that any standard form of therapy is sufficient. This is far from true. It is essential first to know the species involved because they differ markedly in their behavior and response to different drugs. For example, as far as is known, the use of any antimalarial drug will result in the cure of a *falciparum* infection since it is usually of short duration and self limited. On the other hand, quartan malaria may be effectively curbed during the acute phase, yet it seems to adjust itself and survive for years

regardless of the method of treatment. Not only are species important but also the recognition of strains within species can determine the success of therapy. The *vivax* malaria of southern United States responds well to therapy and has a relatively low relapse rate, yet the majority of patients infected with the morphologically identical *vivax* strain from the South Pacific will relapse repeatedly under the same treatment. Also, the response to therapy will vary greatly according to the duration of infection. For example, in mosquito induced *vivax* malaria in luetic patients it takes only a fraction of the amount of the drug to interrupt an acute infection after the sixth to the tenth chill compared to the amount necessary to produce the same effect during its initial stages. Actually most strains of *vivax* will recover spontaneously if allowed to go untreated. Native populations in highly endemic areas require only minimal amounts of a drug to interrupt an acute attack.

It is most important to appreciate the role that immunity plays in the therapy of this disease. It has been demonstrated experimentally in both man and animal that immunity following infection is of very short duration after all parasites have been eradicated. If the physician is using a suppressive drug, he can expect relapses following therapy and should so inform the patient. Curative drugs in endemic areas will only protect the patient temporarily since reinfection can and does occur.

If generalizations about the therapy of malaria are permitted, they should be to use the suppressive drugs in areas where the risks of reinfection are high. This would apply primarily to laborers and military personnel where the administration of the drug must be practical as well as effective. For those in non-endemic areas or even those in endemic areas where exposure is limited, the aim of therapy should be eradication.

QUININE AND RELATED ALKALOIDS

In spite of the great promise of the new compounds, any discussion of the treatment of malaria must start with quinine and related alkaloids. The importance of quinine, past and present, cannot be overemphasized. For years it was regarded as the only truly specific medication for any infectious disease. However, Lourie (1) in 1934 and others showed in experimental malaria of birds that the relapse rate following the maximum amount of tolerated quinine was no less than that of birds recovering spontaneously. This was confirmed in experimental monkey malaria. Animals treated either with quinine or immune serum survived, but retained infections in a chronic form that could not be differentiated. Studies on naturally induced malaria as a treatment for lues of the central nervous system provided an opportunity to prove that quinine in man was not a complete plasmodicide since it did not influence the relapse rate.

Further, when used as a suppressive, it will not prevent the acquisition of an infection although it will effectively control all clinical manifestations. The reason for the many controversies about the efficacy of quinine over the years was probably a failure to recognize variations in the biological behavior of various strains of parasites in response to the drug.

During World War II there were relatively few advances in the improvement of quinine therapy. The synthesis of quinine in 1944 was of academic interest but the process was so difficult and the yield so small that it is of little practical importance.

The other principal cinchona alkaloids, quinidine, cinchonine, and cinchonidine, all possess antiprotoplasmic properties. Their toxicity or unavailability preclude them as practical therapeutic agents. Shannon (2) in 1945 showed that the action of each in a standardized infection was dependent upon its concentration in the plasma. Totaquina, a mixture containing all of the alkaloids of cinchona bark, has been advocated by some primarily because it is cheap or because crude preparations could be used from the bark of low yielding cinchona trees during the period of quinine shortage. Totaquina has not gained favor as a remedy for the treatment of malaria.

The oral administration of quinine salts is always preferable. Various dosages are recommended usually following individual preference or regional customs. One gram (grains XV) three times daily for two days, then 0.6 gram (grains X) for five days is usually sufficient. With any dosage therapy should not be discontinued until the patient has had five days of normal temperature and negative blood smears. Any failures with the above regime are due to the lack of retention or absorption of the drug. In these instances diluted intravenous quinine-dihydrochloride should be given, 0.3 gram (grain V) in 10 cc. of a physiological solution every five to six hours until the patient can tolerate the drug by mouth. The intramuscular use of soluble quinine frequently causes sloughing of tissues and should be avoided.

Quinacrine hydrochloride (Atabrine di-hydrochloride)

Like quinine, quinacrine has a bibliography of enormous proportions. It was discovered in 1933 following an intensive search for synthetic quinine substitutes. It has been amply tried and found to be as successful as quinine for the suppression and treatment of malaria, but it will cure only *falciparum* malaria. When quinine became practically unavailable during World War II, quinacrine met all the demands. The complaints about its ineffectiveness or toxic manifestations usually had their origin in lack of experience.

The chief disadvantage of quinacrine is the discoloration and gastric irritation it produces in many patients.

A course of treatment for quinacrine is 0.2 gram (grain III) every six hours for five doses followed by a 0.1 gram three times a day for six days. For suppression the dosage is 0.1 gram daily in heavily endemic areas. If parenteral medication is necessary, quinacrine should be given intramuscularly in daily doses of 0.2 gram.

4-Aminoquinoline Drugs

Of the many new compounds investigated during the past few years, the 4-aminoquinoline drugs appear to be the most promising, particularly chloroquine (Aralen) = 7-chloro-4-(4-diethylamino-1-methylbutylamino quinoline) and amo-

diaquine (Camoquin hydrochloride) = 4(3-diethylaminomethyl-4-hydroxy-anilino)7-chloroquinoline dihydrate. They possess many advantages over quinine or quinacrine. They are less toxic, provide rapid symptomatic relief for the acute attack, and are highly effective as suppressive agents against *falciparum*, *vivax*, and *malariae* infections. In experimental animals their toxicity is appreciably less than that observed with other antimalarial agents when related to the amount necessary for therapeutic activity (3, 4, 5, 6, 7). When given to man in recommended dosages, both compounds are well tolerated. Chloroquine, the most thoroughly studied, has been subjected to a very critical trial and has now been approved by the Armed Forces for routine use for the troops. Both chloroquine and amodiaquine can be given intramuscularly without difficulty (8, 9, 10) if there is need for parenteral therapy.

As suppressive agents a single weekly dose will prevent the occurrence of clinical symptoms. Chloroquine is being used routinely by the Armed Forces in Korea at a dosage of 0.5 gram of the salt (0.3 gram base) once weekly. Where rigid discipline is enforced, acute malaria has been no problem although the incidence of malaria in the area has been high. Upon leaving the endemic region relapsing *vivax* malaria appears soon after the discontinuance of chloroquine therapy. The Surgeon General of the U. S. Public Health Service reported in September 1951 that over 800 cases of malaria had occurred in recent weeks in returning military personnel. Several investigators have used chloroquine and amodiaquine in civilian populations and have reported no serious toxic manifestations, but a high degree of suppressive and therapeutic activity. For suppression the dosage of chloroquine is 0.5 gram salt (0.3 grams base) given once weekly. The therapeutic dosage is 1 gram (0.6 gram base) as the initial dose followed every six hours by 0.5 gram (0.3 gram base) until a total of 2.5 grams (1.5 grams base) is given. The dosage recommended for amodiaquine for suppression is three tablets (0.6 gram) every two weeks. The dosage for the clinical attack is 0.6 gram (three tablets) taken as a single dose. Thus far this has been found in all instances to be sufficient. However, it is recommended that the normal adult receive one tablet (0.2 gram) twice daily for three days thereafter.

A review of results to date, many of which are still not published, leaves one clearly with the impression that these two compounds are superior agents for the suppression of clinical malaria or the treatment of the acute attack, but neither can be relied upon to eradicate quartan or *vivax* infections. Since these drugs are still in their developmental stages, they are still too expensive for widespread usage, particularly for those in the lower economic groups. As with the older remedies, many years of intensive study will be required before the true usefulness of these compounds is firmly established.

Paludrine (Chlorguanide, Proguanil) N_1-(p-chlorophenyl)-N_5-isopropyl biguanide

Paludrine was discovered by British investigators during the war and was found to be a non toxic compound with considerable antimalarial activity. The chief advantage of this compound against all forms of malaria is the wide margin of safety between the amount necessary to produce toxic symptoms and that

required for therapeutic action. In *falciparum* malaria it is a causal prophylactic and usually cures (11, 12). It is an effective suppressant against *vivax* infections. However, there is an ever increasing number of reports showing that many *vivax* strains are becoming drug resistant. This was recognized early in experimental animals, and probably represents the first authentic instance of drug resistance by a malarial parasite (13, 14). This was also found true with *P. falciparum* and would seem to remove a great measure of its future usefulness as a suppressive compound. Van Goor (15) and associates reported results of one year's prophylaxis in Java with chlorguanide and chloroquine that showed a definite resistance of *P. falciparum* and *P. malariae* to the former drug. Seaton and Adams (16) isolated a strain of *P. falciparum* from a patient who had been taking chlorguanide occasionally, and the strain was passed through a series of neurosyphilitics on a low maintenance dosage of chlorguanide. At the seventh passage the strain was resistant to a dosage of 1,000 miligrams a day for ten days. It is of interest to note that the strain maintained its resistance to chlorguanide after passage through the mosquito. Field and Edeson (17) found that with certain *falciparum* infections a single tablet of 100 milligrams was enough to obtain a clinical cure. However, in the same locality two years later one out of every four cases failed to respond at two to three times this dose. These patients were drawn from a population taking suppressive chlorguanide. They also reported one case in which a dosage one hundred times greater than that required to produce a therapeutic result in a normal case failed to cause the disappearance of parasites for more than a few days. Another disadvantage is its inability to produce a normal temperature or cause parasites to disappear promptly when used for the acute attack. In some instances fever may persist for five to seven days after the commencement of therapy and as such it limits its usefulness. There is an increasing number of recommendations that quinine or quinacrine be given in combination with chlorguanide to eliminate this disadvantage. A careful scrutiny of all the literature, however, which cannot be detailed in this report, seems to indicate that the 4-aminoquinoline drugs are superior to chlorguanide since they have not yet at least been shown to produce resistant strains and they act very promptly and efficiently. On the other hand, chlorguanide is a much cheaper drug, and in this respect it possesses a real advantage over the 4-aminoquinoline compounds.

Pentaquine 6-methoxy-8-(5-isopropylaminoamylamino) quinoline

When the recent search for a curative drug effective for all types of malaria failed to reveal any new compounds, greater attention was given to pamaquine. This drug, commonly known as Plasmochin, was discovered in 1927, and a decade of experience was summarized by the Malaria Committee of the League of Nations, (18). It was shown to be useful in reducing relapse rates, but toxic manifestations prevented widescale usage. However, in recent studies, it was most encouraging to find that some of its analogues were less toxic and could effect radical cure. Pentaquine, first reported by Loeb, *et al.* (19) in 1946 had a pharmacological activity closely parallel to pamaquine in experimental ani-

mals, but was more effective against various infections in lower animals. Alving and coworkers (20) in 1948 reported it significantly superior as a therapeutic agent in man. In their first report, susceptible volunteers were infected with varying numbers of *vivax* sporozoites (Chesson strain). Moderate infections treated with 60 milligrams of pentaquine base daily and 2.0 grams of quinine sulfate had a relapse rate of 2% as compared with 67% in the controls. In severe infections the relapse rate was 18% in the treated group while 98% of the controls relapsed. These and many other studies confirmed the findings that nontoxic doses of pentaquine possessed a high curative value against *vivax* malaria. The author (21) treated 183 exservicemen suffering from recurring *vivax* malaria. Most had acquired their original infection in the South Pacific and had been found to be extremely refractory to all other treatment. Pentaquine therapy was initiated only during an acute relapse or with demonstrable parasites in blood smears. The dose consisted of pentaquine, 30 milligrams, and quinine sulfate, 2.0 grams, for fourteen days. Patients were not hospitalized for treatment. The results after a two year follow-up showed 163 of the 183 to be cured. A recent questionnaire at the end of five years did not reveal any history of additional relapses. Pentaquine is now available commercially in combination with quinine in tablet form. The dose is one tablet (0.3 gram) t.i.d. daily for fourteen days.

During a continued search for improved compounds, Elderfield synthesized primaquine, 8-(4-amino-1-methyl butylamino)-6-methoxy quinoline. Extensive studies by Schmidt showed that the toxicity of several 8-amino compounds in lower animals were of the same order of magnitude. However, Edgcomb and coworkers (22) reported that primaquine was superior to pentaquine in man because it would cure severe infections of *vivax* malaria in doses that were relatively non toxic. The estimated dosage for one hundred per cent cure was only 22.5 milligrams base as compared to 100 milligrams of pentaquine. Within the past year many studies, yet unreported, by Alving and coworkers at the University of Chicago and Coatney and Cooper and colleagues at the U. S. Public Health Service, using volunteers in prisons, show beyond doubt that primaquine given with chloroquine or quinine will cure the most persistently relapsing types of *vivax* malaria known.

Alving, Arnold and Robinson in 1951 subjected primaquine to a most critical test for toxicity when it was given to 2,300 servicemen, on troop transports from Japan, in a curative dosage of 15 milligrams base, with relatively no difficulty. It was found that an occasional Negro developed mild to moderate anemia with primaquine. However, Thaeler *et al.* (23) in 1951 gave it to 371 malnourished natives of Nicaragua, children and adults, and had no difficulty with dosages below 30 milligrams base per 150 pounds body weight.

Of greatest interest and importance was the finding of Arnold *et al.* (24) in 1951 that primaquine acted as a true causal prophylactic against the bites of ten mosquitoes heavily infected with *P. vivax*. Pamaquine given at 90 milligrams base in the same experiment did not protect three out of five infections. Primaquine on the other hand, in six daily doses of 30 milligrams base, protected all

ten men tested. Further tests showed that a single dose of 30 milligrams of primaquine base given only twice weekly would prevent *vivax* malaria in eight of ten subjects.

Conclusion

For the treatment of acute attacks of malaria, chloroquine, a 4-amino quinoline compound, seems to be the drug of choice. Amodiaquine (Camoquin) appears to be equally promising from the available data although it has not as yet been evaluated as extensively as chloroquine. Likewise these same compounds are the most effective suppressants.

For the cure of malaria, particularly relapsing *vivax* infections, primaquine given in dosages of 15 milligrams base daily for two weeks concurrently with quinine or chloroquine has proven to be more reliable than any therapeutic regime yet reported. This compound is available only for experimental purposes at the present time. Pentaquine, although somewhat inferior to primaquine, is obtainable and on the basis of results obtained is recommended for the treatment of recurring *vivax* malaria when radical cure is indicated.

REFERENCES

In future issues of this Journal, references will be cited by date and not by number.

1. Lourie, E. M., Studies on chemotherapy in bird malaria, *Ann. Trop. Med.* **28:** 151, 1934.
2. Shannon, J. A., and Earle, D. P., Jr., Recent advances in the treatment of malaria, *Bull. New York Acad. Med.* **21:** 467, 1945.
3. Wiselogle, Frederick Y., Editor, A Survey of Antimalarial Drugs—1941–1945, Ann Arbor, Michigan, J. W. Edwards, 1946.
4. Loeb, R. F., *et al.*, Activity of new antimalarial agent chloroquine, *J. Amer. Med. Assoc.* **130:** 1069–1070, 1946.
5. Burckhalter, J. H., *et al.*, Aminoalkylphenols as antimalarials. The synthesis of Camoquin, *J. Am. Chem. Soc.* **70:** 1363, 1948.
6. Berliner, R. W., *et al.*, Studies on chemotherapy of the human malarias. VI. The physiological disposition, antimalarial activity and toxicity of several derivatives of 4-aminoquinoline, *J. Clin. Investigation* **28:** 98–107, 1948.
7. Schmidt, L. H., On the pharmacology of the 4-aminoquinolines. A survey of antimalarial drugs, 1941–45, Ann Arbor, Michigan, J. W. Edwards, **1:** 94–106, 1946.
8. Ejercito, H., and Duque, M., Preliminary report on CAM-AQI dihydrochloride (Miraquin Camoquin) in the treatment of human malaria, *J. Philippine M. A.* **24:** 833, 1948.
9. Culwell, W. B., Cooper, W. C., White, W. C., Lintz, H. A., and Coatney, G. R., Studies in human malaria. XX. The intramuscular injection of chloroquine, *J. Nat. Malaria Soc.* **7:** 311–315, 1948.
10. Payne, E. H., Sharp, E. A., and Nickel, K. C., Parenteral use of Camoquin hydrochloride as an antimalarial, *Am. J. Trop. Med.* **29:** 353, 1949.
11. Fairley, N. H., *et al.*, Researches on Paludrine (M 4888) in malaria. An experimental investigation undertaken by the L. H. Q. Medical Research Unit, *Tr. Roy. Soc. Trop. Med. & Hyg.* **40:** 105–151, 1949.
12. Covell, G., Nichol, W. D., Shute, P. G., and Maryon, M., "Paludrine" (Proguanil) in prophylaxis and treatment of malarial infections caused by a West African strain of *Plasmodium falciparum*, *Brit. M. J.* **1:** 88–91, 1949.

13. Bishop, A., and Birkett, B., Acquired resistance to Paludrine in *Plasmodium gallinaceum:* acquired resistance and persistence after passage through the mosquito, *Nature* **159:** 884–885, 1947.
14. Thompson, P. E., On the ability of *Plasmodium lophurae* to acquire resistance to chlorguanide, Camoquin, and chloroquine, *J. Infect. Dis.* **83:** 250–255, 1948.
15. van Goor, W. T., Lodens, J. G., and Gomez, J. A., Clinical prophylaxis with Proguanil and Nivaquine in a community in Java, *Documenta Neerlandica et Indonesica de Morbis Tropicis*, Amsterdam, **2:** 341–349, 1950.
16. Seaton, D. R. and Adams, A. R. D., Acquired resistance to Proguanil in *Plasmodium falciparum*, Lancet 323–324, 1949.
17. Field, J. W. and Edeson, J. F. B., Paludrine resistant *falciparum* malaria, *Tr. Roy. Soc. Trop. Med. & Hyg.* **43:** 233–236, 1949.
18. Fourth General Report of the Malaria Commission, *Bull. of the Health Org. of the League of Nations* **6:** 895, 1937.
19. Loeb, R. F., Activity of a new antimalarial agent, pentaquine (Sn-13276), *J. Amer. Med. Assoc.* **132:** 321, 1946.
20. Alving, Alf S., *et al.*, Pentaquine (Sn-13276), a therapeutic agent effective in reducing the relapse rate in *vivax* malaria, *J. Clin. Invest.* **27:** 25–33, 1948.
21. Coggeshall, L. T. and Rice, F. A., Cure of chronic *vivax* malaria with pentaquine, *J. Amer. Med. Assoc.* **139:** 437–438, 1949.
22. Edgcomb, John H., *et al.*, Primaquine (Sn-13272), a new curative agent in *vivax* malaria. A preliminary report, *J. Natl. Mal. Soc.* **9:** 285–292, 1950.
23. Thaeler, A. David, Arnold, John D., and Alving, Alf S., Field studies of primaquine in Nicaragua (in press) read at National Malaria Society meeting, Chicago, 1951.
24. Arnold, John D., Hockwald, Robert S., Clayman, Charles B., Alving, Alf S., Prophylactic studies with primaquine (in press) read at National Malaria Society, Chicago, 1951.

THE *IN VITRO* ACTIVITY OF CERTAIN ANTIMALARIALS AGAINST ERYTHROCYTIC FORMS OF *PLASMODIUM GALLINACEUM*[1]

D. JANE TAYLOR, EDWARD S. JOSEPHSON, JOSEPH GREENBERG, AND G. ROBERT COATNEY

National Institutes of Health, National Microbiological Institute, Bethesda 14, Maryland

As a result of the coordinated malaria drug-testing program in the United States inagurated early in World War II, over 16,000 compounds were tested for antimalarial activity and several new compounds were found to be practical as antimalarials. The emphasis in malaria chemotherapy shifted in the post-war period from the mass screening of compounds to a study of the mode of action of selected drugs in the attempt to develop more effective agents. Such studies were complicated by drug toxicity and by the metabolic activities of the vertebrate hosts wherein the drugs were subjected to localization and storage in tissues, to varying rates of excretion, and to degradation to either more active or less active metabolic intermediates.

In order to study the mode of action of antimalarials without the complications introduced by the vertebrate host, a recently described technique for the appraisal of antimalarial activity *in vitro* against *Plasmodium gallinaceum* (1, 2) was applied to a series of compounds of known antimalarial activity *in vivo*. The drugs tested and reported here are quinine; quinacrine (6-chloro-2-methoxy-9-[4-diethylamino-1-methylbutylamino]acridine); chloroquine (7-chloro-4-[4-diethylamino-1-methylbutylamino]quinoline); amodiaquin (Camoquin) (7-chloro-4-[3-diethylaminomethyl-4-hydroxyanilino]quinoline); sulfadiazine; metachloridine (N^1-[5-chloro-2-pyrimidyl]metanilamide); pamaquine (8-[4-diethylamino-1-methylbutylamino]-6-methoxyquinoline); DR 16,070 (8-[4-diethylamino-1-methylbutylamino]-5,6-quinolinequinone) isolated from pamaquine-treated chickens (4); 2,4-diamino-5-*p*-chlorophenoxy-6-methyl-pyrimidine; 2,4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine; 2,4-diamino-5-*p*-nitrobenzyl-6-metlylpyrimidine; 2,4-diamino-5-*p*-chlorophenyl-6-*n*-amylpyrimidine; 2,4-diamino-6,7-diphenylpyrimido(4,5-b)pyrazine; SN 12,320 (2-[3-(decahydro-2-naphthyl)-propyl]-3-hydroxy-1,4-naphthoquinone); chlorguanide (N^1-*p*-chlorophenyl-N^5-isopropylbiguanide) and two metabolic intermediates of chlorguanide isolated from humans and monkeys, N^1-*p*-chlorophenylbiguanide (18) and 2-amino-4,4-dimethyl-6-(*p*-chlorophenylamino)-3,4-dihydro-1,3,5-triazine (17).

METHODS

The details for the *in vitro* maintenance of the erythrocytic forms of *P. gallinaceum* and appraisal of antimalarial activity against this parasite have been described elsewhere (1, 2). In these tests 5×10^8 parasitized chick erythrocytes were rocked with the test drug in a whole chick blood medium at 37°C. for 24 hours in rocker-dilution boats. The minimum effective concentration of a drug

[1] Presented at the XIIth International Congress of Pure and Applied Chemistry, New York, New York, September 10–13, 1951.

was that amount which under the test conditions prevented 90 per cent of the parasites initially present in the culture from reproducing when subinoculated into week-old chicks. This corresponds to a 1.3-day increase in the mean prepatent period of the infection in chicks inoculated with parasites exposed to drug over that of those inoculated with control cultures.

Crude concentrates of mixtures of metabolic intermediates of pamaquine from tissues and droppings of chickens treated with a single oral dose of pamaquine (20 mg./kg. body weight) were obtained by extraction into ethylene dichloride and then into a small volume of 0.1 N HCl. The droppings were collected over an 18-hour period following drug administration and the tissue extracts were obtained one to two hours after drug administration. The pamaquine content in such preparations was determined by the method of Brodie *et al.* (3). Details for the isolation and identification of one of the metabolic intermediates of pamaquine, DR 16,070, are described by Josephson *et al.* (4, 5).

TABLE 1

Differences between specific and non-specific methods for estimation of chloroquine, quinacrine, and quinine in whole chicken blood

COMPOUND	SPECIFIC	NON-SPECIFIC	REFERENCE
Chloroquine	Extract with heptane followed by 3 washes with 0.1 N NaOH	Extract with ethylene dichloride. No alkaline washes	Brodie *et al.* (7)
Quinacrine	Extract with petroleum ether followed by extraction into acid	Extract with ethylene dichloride. Measure drug in acidified ethylene dichloride	Brodie *et al.* (8)
Quinine	Extract with benzene and then with 0.1 N H_2SO_4	Measure directly in diluted protein-free filtrate	Brodie *et al.* (8)

The minimum effective concentrations *in vivo* of antimalarials in whole blood and plasma were obtained from chickens treated twice daily for four days at the minimum effective dose as determined by the method of Coatney and Sebrell (6). Drug measurements were performed on samples drawn two hours after the last dose. Chloroquine was assayed by the method of Brodie *et al.* (7); quinacrine, by the method of Brodie *et al.* (8); quinine, by the method of Brodie *et al.* (8); SN 12,320, by a colorimetric method developed by Brodie and modified by Butler (outlined in Wiselogle (9)); chlorguanide, by the method of Spinks and Tottey (10); and sulfadiazine and metachloridine, by the method of Bratton and Marshall (11).

The extent of degradation of drugs *in vitro* during 24 hours was studied by comparing drug concentrations as determined (1) by a method specific for the parent drug alone at the zero hour and at 24 hours of incubation, and (2) by non-specific methods which estimate both parent drug and metabolites. The differences between the specific and non-specific methods for the estimation of chloroquine, quinacrine, and quinine are indicated in table 1.

RESULTS

Data obtained with chloroquine, quinacrine, quinine, and SN 12,320 against *P. gallinaceum in vitro* and *in vivo* are shown in table 2. On the basis of drug concentrations in whole chicken blood, the antimalarial activity of these compounds *in vitro* and *in vivo* are in close agreement.

The comparative activities based upon the concentrations in plasma of a series of antimalarials against *P. gallinaceum* in the chick and those reported

TABLE 2

The antimalarial activity of chloroquine, quinacrine, quinine, and SN 12,320 against Plasmodium gallinaceum in vitro *and* in vivo

DRUG	MINIMUM EFFECTIVE CONCENTRATION (MG./l. WHOLE CHICKEN BLOOD)		DEGRADATION *in vitro* IN 24 HOURS
	In vitro	*In vivo*	
Chloroquine	0.25	0.2	none*
Quinacrine	0.50	1.1	none*
Quinine	2.0	1.0	none*
SN 12,320	6.25	9.0	not assayed

* Within limitations of methods of assay.

TABLE 3

The antimalarial activity of test drugs against Plasmodium gallinaceum *in the chick and* Plasmodium vivax *in man*

DRUG	M.E.C. *in vivo* (MG./l. PLASMA)	
	P. gallinaceum	*P. vivax*
Chloroquinine	0.036	0.010 (Berliner *et al.*, (26))
Quinacrine	0.018	0.025 (Berliner *et al.*, (26))
Quinine	1.0	2.0 (Taggart *et al.*, (27))
SN 12,320*	7.0	—
SN 8,557*	—	43–61 (Ruhe *et al.*, (28))
Chlorguanide	0.02	<0.01 (Earle *et al.*, (29))
Sulfadiazine	34†	56–151† (Wiselogle, (9))
Metachloridine	<10†	1–9† (Wiselogle, (9))

* 2-[3-(Decahydro-2-naphthyl)propyl]-3-hydroxy-1,4-naphthoquinone. SN 8,557 is a mixture of stereoisomers; SN 12,320 is the isomer melting at 126°C.

† Whole blood.

in the literature for *P. vivax* in man are indicated in table 3. In general the antimalarial activity of these drugs is the same *in vivo* against both parasites. Data on SN 12,320 against *P. vivax* in man were not found in the literature; hence, it is not known how much of the activity in humans of SN 8557, which is a mixture of stereoisomers, can be attributed to the isomer SN 12, 320.

In table 4 and 5 data are presented which indicate that the sulfonamides, sulfadiazine and metachloridine, and a series of compounds possessing the

TABLE 4

The antimalarial activity of sulfadiazine, metachloridine, chlorguanide, and two metabolic intermediates of chlorguanide from the monkey against Plasmodium gallinaceum

DRUG	MINIMUM EFFECTIVE CONCENTRATION (MG./L. WHOLE BLOOD)	
	In vitro	*In vivo*
Sulfadiazine	>750	34
Metachloridine	>800	<10
Chlorguanide	50	0.11
N^1-*p*-chlorophenylbiguanide	>100	—
2-amino-4,4-dimethyl-6-(*p*-chlorophenylamino)-3,4-dihydro-1,3,5-triazine	>100	—

TABLE 5

The in vitro *ineffectiveness of a series of "biguanide-type" compounds and sulfonamides possessing antimalarial activity against* Plasmodium gallinaceum in vivo

DRUG	M.E.C. *in vitro* (MG./L. WHOLE BLOOD)	M.E.D. *in vivo* (MG./KG.)
Chlorguanide	50	1.5
2,4-diamino-5-*p*-chloro-phenoxy-6-methylpyrimidine	75 (?)	8
2,4-diamino-5-*p*-chloro-phenyl-6-ethylpyrimidine	>100	0.016
2,4-diamino-5-*p*-nitrobenzyl-6-methylpyrimidine	>100	0.5
2,4,-diamino-5-*p*-chloro-phenyl-6-*n*-amylpyrimidine	>100	<0.125
2,4,-diamino-6,7-diphenyl-pyrimido(4,5-b)pyrazine	>100	16
Sulfadiazine	>750	30
Metachloridine	>800	2

TABLE 6

Effects of pamaquine and its metabolic intermediates derived from chickens against Plasmodium gallinaceum in vitro

TEST MATERIAL	*In vitro* (MG./L.)
M.E.C. of pamaquine	10
Pamaquine in active preparations of chicken	
(a) Tissue homogenates	≦2.9
(b) Whole blood	0.25
(c) Droppings	≦0.35
M.E.C. of 8-(diethylamino-1-methylbutylamino)-5,6-quinolinequinone from pamaquine-treated chickens (DR 16,070)	0.625

biguanide group are relatively ineffective against *P. gallinaceum in vitro* although some of them are very active against this parasite *in vivo*. The two metabolic intermediates of chlorguanide isolated from monkeys, N^1-*p*-chlorophenylbi-

guanide (18) and 2-amino-4,4-dimethyl-6-(*p*-chlorophenylamino)-3,4-dihydro-1,3,5-triazine (17), were less active *in vitro* against *P. gallinaceum* than the parent chlorguanide.

From the data presented in table 6 it is apparent that pamaquine is much less active *in vitro* against *P. gallinaceum* than some of its metabolic intermediates.

DISCUSSION

The compounds in this report fall into two categories: (1) those which exhibited antimalarial activity *in vitro* in concentrations approximating those found in the blood of adequately treated chicks, and (2) those which were inactive *in vitro*, or were active in concentrations far in excess of concentrations effective *in vivo*.

Chloroquine, quinine, and quinacrine were directly plasmodicidal, since the minimum effective concentrations in blood were approximately the same *in vitro* as *in vivo* and there was no apparent degradation of these compounds in a whole blood medium over the 24-hour test period. Geiman (12) and McKee (13) reported that these compounds were directly active against *P. cynomolgi in vitro*, whereas chlorguanide and pentaquine were inactive and isopentaquine only slightly active. The naphthoquinone, SN 12,320, was probably also directly active because there was good correlation between effective blood levels *in vitro* and *in vivo*. However, no tests were made to determine whether this compound was degraded in our whole blood medium during the 24-hour test period. Amodiaquin, a 4-aminoquinoline related in chemical structure to chloroquine, was found to be effective *in vitro* when introduced into the cultures at a concentration of 0.125 mg./l. Since amodiaquin was completely degraded in 24 hours in the cultures, it is not possible to state whether antimalarial activity should be attributed to the parent compound, its metabolites, or both.

The remaining compounds tested were essentially inactive *in vitro* at concentrations associated with antimalarial activity *in vivo*. The antimalarial activity which chlorguanide exhibited at very high concentrations differed qualitatively from that observed *in vivo* for the following reasons: A strain of *P. gallinaceum* 16-fold resistant to chlorguanide *in vivo* was as sensitive as a normal strain to high levels of chlorguanide *in vitro*. Although the antimalarial activity of chlorguanide *in vivo* could be potentiated by sulfadiazine (14) and inhibited by folic acid (15), the antimalarial activity *in vitro* was not influenced by either sulfadiazine or folic acid.

There seem to be two ways to explain the inactivity of certain antimalarials in our *in vitro* cultures: (1) The compounds themselves may have been inactive but their activity could have been mediated through a product of their degradation by the host; or (2) the compounds may have failed to demonstrate activity because of abnormalities of the parasites in the test system.

The first explanation appears to be adequate for the failure of pamaquine to exhibit antimalarial activity *in vitro*. Concentrates from tissues and droppings of chickens treated with this compound showed *in vitro* antimalarial activity, though they contained amounts of the parent compound known to be inef-

fective. Furthermore, DR 16,070, a product of the degradation of pamaquine by the chicken, has been isolated, identified, and found to be 16-fold more active than the parent substance (4, 5). There was no significant degradation of pamaquine in our culture medium during the period of the test.

Hawking (16) has reported that chlorguanide itself is inactive against the exoerythrocytic forms of *P. gallinaceum*, but is activated by the chick host *in vivo* or by chick liver *in vitro*. McKee (13) reported this to be true of chlorguanide against *P. cynomolgi in vitro*. However, neither the triazine (17) nor the N^1-*p*-chlorophenylbiguanide (18), isolated from monkeys and humans treated with chlorguanide, had any activity against *P. gallinaceum in vitro*. Against *P. cynomolgi* in the monkey the triazine was inactive (17) and the N^1-*p*-chlorophenylbiguanide was no more than $\frac{1}{64}$ as active as the parent chlorguanide (18). These observations still do not preclude the possibility that the activity of chlorguanide could be attributed to a metabolic intermediate which occurred in minute quantities or which was highly unstable. It is, however, pertinent that blood of chicks drawn two to three hours after the last dose (32 mg./kg.) of chlorguanide administered twice daily for four days was inactive in our *in vitro* cultures. This is true also of the diaminopteridine, 2,4-diamino-6,7-diphenylpyrimido (4,5-b)pyrazine.

We attribute the inactivity of chlorguanide and the other biguanide-type compounds to the fact that they are plasmodistatic and not plasmodicidal. It was shown in an earlier paper (1) that the parasites in our cultures, while not static, did not multiply. They were unable to advance beyond the stage of early schizonts, which implies that they could not significantly increase the amounts of nuclear material. Black (19) observed that sulfadiazine and chlorguanide exert their inhibitory effects on *P. falciparum in vitro* during schizogony. It is well known that the sulfonamides exert their effects on various organisms by inhibiting their reproduction. Furthermore, there is no suggestion that sulfonamides require degradation by the host in order to exert activity. Sulfadiazine was completely without inhibitory effect in our system even at very high concentrations. It might be well to point out that certain similarities in mode of action exist among sulfonamides and the "biguanide-type" compounds and between certain biguanides and chlorguanide (20, 21). For example, all of the "biguanide-type" compounds are potentiated by sulfonamides *in vivo* (14, 22, 23) all of them are partially inhibited by folic acid *in vivo* (15, 22); and some of them exhibit cross-resistance with sulfonamides (24, 25). It is therefore reasonable to assume that all these compounds are plasmodistatic and would not exert inhibitory effects in our non-multiplying cultures. Whether some or all of these compounds require prior degradation to active metabolites cannot be answered on the basis of our evidence.

SUMMARY

A series of compounds was tested for antimalarial activity *in vitro* against the erythrocytic forms of *Plasmodium gallinaceum*. Chloroquine, quinacrine, quinine, and the naphthoquinone, SN 12,320, were active at concentrations approxi-

mating those found in the blood of adequately treated chicks. Pamaquine was relatively inactive *in vitro*, but when administered to chickens gave rise to active metabolic intermediates. Sulfadiazine, metachloridine, chlorguanide, and two of its metabolic intermediates from monkeys, a number of 2,4-diaminopyrimidines, and a 2,4-diaminopterin were relatively inactive *in vitro*.

ACKNOWLEDGMENTS

We are grateful to the following for furnishing test compounds: Dr. Louis F. Fieser, Harvard University, Cambridge, Massachusetts for SN 12,320; Dr. Sterling Brackett, American Cyanamid Company, New York, New York for sulfadiazine and metachloridine; Dr. Leon H. Schmidt, The Christ Hospital, Cincinnati, Ohio for N^1-*p*-chlorophenyl-biguanide and 2-amino-4,4-dimethyl-6-(*p*-chlorophenylamino)-3,4-dihydro-1,3,5-triazine; Dr. George H. Hitchings The Wellcome Laboratories, Tuckahoe, New York for the diaminopyrimidines; and Dr. Cornelius K. Cain, Cornell University, Ithaca, New York for 2,4-diamino-6,7-diphenylpyrimido(4,5-b)pyrazine.

The technical assistance of George W. Rusten, Sr., Anne P. Jowett, Edna M. Richeson, Margaret G. Johnson, John Phillips, Louise V. Perkins, Nancy K. Allen, and Elizabeth A. Brown is gratefully acknowledged.

REFERENCES

In future issues of this Journal, references will be cited by date and not by number.

1. Taylor, D. J., Greenberg, J., Josephson, E. S., and Ray, A. P., Studies on *Plasmodium gallinaceum in vitro*. I. A method for maintenance of the erythrocytic parasite *in vitro*. *J. Infect. Dis.* **88**: 158–162, 1951.
2. Greenberg, J., Taylor, D. J., and Josephson, E. S., Studies on *Plasmodium gallinaceum in vitro*. II. The effects of some 8-aminoquinolines against the erythrocytic parasites, *J. Infect. Dis.* **88**: 163–167, 1951.
3. Brodie, B. B., Udenfriend, S., and Taggart, J. V., The estimation of basic organic compounds in biological material. IV. Estimation by coupling with diazonium salts, *J. Biol. Chem.* **168**: 327–334, 1947.
4. Josephson, E. S., Greenberg, J., Taylor, D. J., and Bami, H. L., A metabolite of pamaquine from chickens, *J. Pharmacol. & Exper. Therap.* **103**: 7–10, 1951.
5. Josephson, E. S., Taylor, D. J., Greenberg, J., and Ray, A. P., A metabolic intermediate of pamaquine from chickens, *Proc. Soc. Exper. Biol. & Med.* **76**: 700–703, 1951.
6. Coatney, G. R., and Sebrell, W. H., In F. Y. Wiselogle's "A survey of antimalarial drugs, 1941–1945." J. W. Edwards, Ann Arbor, Michigan, 1946.
7. Brodie, B. B., Udenfriend, S., Dill, W., and Chenkin, T., The estimation of basic organic compounds in biological material. III. Estimation by conversion to fluorescent compounds, *J. Biol. Chem.* **168**: 319–325, 1947.
8. Brodie, B. B., Udenfriend, S., Dill, W., and Downing, H., The estimation of basic organic compounds in biological material. II. Estimation of fluorescent compounds, *J. Biol. Chem.* **168**: 311–318, 1947.
9. Wiselogle, F. Y., A survey of antimalarial drugs, 1941–1945, J. W. Edwards. Ann Arbor, Michigan.
10. Spinks, A., and Tottey, M. M., Studies on synthetic antimalarial drugs. XV. Hydrolytic determination of paludrine, *Ann. Trop. Med.* **40**: 101–112, 1946.

11. Bratton, A. C., Jr., and Marshall, E. K., Jr., A new coupling component for sulfanilamide determinations, *J. Biol. Chem.* **128:** 537–550, 1939.
12. Geiman, Q. M., Cultivation and metabolism of malarial parasites, *Proc., 4th International Congresses on Trop. Dis. and Malaria* **1:** 618–628, 1948.
13. McKee, R. W., Biochemistry of *Plasmodium* and the influence of antimalarials, *Biochem. and Physiol. Protozoa* **1:** 251–322, 1951.
14. Greenberg, J., Boyd, B. L., and Josephson, E. S., Synergistic effect of chlorguanide and sulfadiazine against *Plasmodium gallinaceum* in the chick, *J. Pharmacol. & Exper. Therap.* **94:** 60–64, 1948.
15. Greenberg, J., Inhibition of the antimalarial activity of chlorguanide by pteroylglutamic acid, *Proc. Soc. Exper. Biol. & Med.* **71:** 306–308, 1949.
16. Hawking, F., Activation of paludrine *in vitro*, *Nature*, London **159:** 409, 1947.
17. Crounse, N. N., Isolation and identification of a metabolite of chlorguanide, *J. Organic Chem.* **16:** 492–500, 1951.
18. Schmidt, L. H., 1949. Personal communication.
19. Bl ack, R. H., The effect of anti-malarial drugs on *Plasmodium falciparum* (New Guinea strains) developing *in vitro*, *Tr. Roy. Soc. Trop. Med. & Hyg.* **40:** 163–170, 1946.
20. Falco, E. A., Hitchings, G. H., Russell, P. B., and VanderWerff, H., Antimalarials as antagonists of purines and pteroylglutamic acid, *Nature*, London **164:** 107, 1949.
21. Falco, E. A., Goodwin, L. G., Hitchings, G. H., Rollo, I. M., and Russell, P. B., 2:4-Diaminopyrimidines—A new series of antimalarials, *Brit. J. Pharmacol.* **6:** 185–200, 1951.
22. Greenberg, J., The antimalarial activity of 2,4-diamino-6,7-diphenylpterin; its potentiation by sulfadizine and inhibition by pteroylglutamic acid, *J. Pharmacol. & Exper. Therap.* **97:** 484–487, 1949.
23. Greenberg, J., and Richeson, E. M., Potentiation of the antimalarial activity of sulfadiazine by 2,4-diamino-5-aryloxypyrimidines, *J. Pharmacol. & Exper. Therap.* **99:** 444–449, 1950.
24. Bishop, A., and McConnachie, E. W., Cross-resistance between sulpharilamide and paludrine (proguanil) in a strain of *Plasmodium gallinaceum* resistant to sulphanilamide, *Parasitology* **40:** 175–178, 1950.
25. Greenberg, J., and Richeson, E. M., Effect of 2,4-diamino-5-(*p*-chlorophenoxy)-6-methylpyrimidine and 2,4-diamino-6,7-diphenylpteridine on chlorguanide-resistant strain of *Plasmodium gallinaceum*, *Proc. Soc. Exper. Biol. & Med.* **77:** 174–176, 1951.
26. Berliner, R. W., Earle, D. P., Jr., Taggart, J. V., Zubrod, C. G., Welch, W. J., Conan, N. J., Bauman, E., Scudder, S. T., and Shannon, J. A., Studies on the chemotherapy of the human malarias. VI. The physiological disposition, antimalarial activity, and toxicity of several derivatives of 4-aminoquinoline, *J. Clin. Investigation* **27,** part **2:** 98–107, 1948.
27. Taggart, J. V., Earle, D. P., Jr., Berliner, R. W., Zubrod, C. G., Welch, W. J., Wise, N. B., Schroeder, E. J., London, I. M., and Shannon, J. A., Studies on the chemotherapy of the human malarias. III. The physiological disposition and antimalarial activity of the cinchona alkaloids, *J. Clin. Investigation* **27,** part 2: 80–86, 1948.
28. Ruhe, D. S., Cooper, W. C., Coatney, G. R., and Josephson, E. S., Studies in human malaria. XIV. The ineffectiveness of colchicine (SN 12,080), SN 7266, and SN 8557 as curative agents against St. Elizabeth strain *vivax* malaria, *Am. J. Hyg.* **49:** 361–366, 1949.
29. Earle, D. P., Jr., Berliner, R. W., Taggart, J. V., Zubrod, C. G., Welch, W. J., Bigelow, F. S., Kennedy, T. J., Jr., and Shannon, J. A., Studies on the chemotherapy of the human malarias. X. The suppressive antimalarial effect of paludrine, *J. Clin. Investigation* **27,** part 2: 130–133, 1948.

MODERN CRITERIA FOR THE LABORATORY DIAGNOSIS OF AMEBIASIS

ERNEST CARROLL FAUST[1]

Department of Tropical Medicine and Public Health, Tulane University, New Orleans, La.

Clinically speaking, the term "amebiasis" refers to human infection with the pathogen *Endamoeba histolytica*. While an experienced physician may obtain considerable evidence that his patient is suffering from amebiasis, his presumptive diagnosis needs laboratory confirmation. Moreover, a large proportion of infected individuals present no clear-cut symptoms referable to this disease and the first evidence is provided when a stool specimen of the patient is sent to the laboratory for microscopic examination. Thus, amebiasis, like brucellosis, depends primarily on the laboratory for its diagnosis.

REQUIREMENTS FOR DEPENDABLE LABORATORY DIAGNOSIS OF AMEBIASIS

1. *Demonstration of Endamoeba histolytica.* Although the time may come (or possibly soon be at hand) when relatively dependable diagnosis of amebiasis can be made by serologic methods, today, just as a quarter of a century ago, proof of infection requires that the ameba itself must be demonstrated under the microscope.

In unformed stools and tissue aspirates *E. histolytica* typically occurs in the trophozoite stage. In the living, active state it will exhibit a characteristic progressive movement of the pseudopodia which the experienced microscopist recognizes to be specifically different from that of other amebas and phagocytic cells. Any cell which fails to demonstrate the motility of *E. histolytica* trophozoites can not be safely diagnosed as *E. histolytica*. *E. histolytica* trophozoites may or may not have red blood cell inclusions, while at times trophozoites of *E. coli* and other intestinal protozoa ingest these cells. Hence their presence in the parasite is not pathognomonic. In formed stools only the cystic stage is encountered, except in flecks of blood or mucus which may be adherent to the feces and may contain nests of trophozoites. At this point it is important to emphasize that there is no substantial evidence that cysts of this ameba have been recovered from aspirates of the intestinal wall, liver, lungs, brain or body cavities.

Material suspected of containing active or encysted stages of *E. histolytica* should first be examined in a drop or two of physiologic salt solution as an unstained cover-glass-mounted film. If trophozoites are present, their motility can be studied; if cysts are revealed, they frequently contain characteristic rod-shaped chromatoidals. Likewise, an iodine-stained wet mount should be made to observe the structural characters of the nuclei (one in the trophozoite stage, one to 4, rarely 8, in the cyst), which have a central karyosome and

[1] The writer is indebted to Doctor Wm. W. Frye for reading the manuscript and making several valuable suggestions.

peripheral chromatin beads just within the nuclear membrane. Usually there is no need to prepare permanent hematoxylin-stained films, but at times these are needed to differentiate the trophozoites from those of other intestinal amebas and to provide a clearer picture of the nuclear characters in the cysts if these organelles are obscured in the wet film by dense deposits of glycogen or masses of chromatoidals. However, if the material submitted for examination contains only objects of doubtful diagnosability, fresh specimens should be requested for study. This is much safer than to hazard a positive diagnosis on unsatisfactory material.

Following direct-film examination the zinc sulfate concentration technic should be carried out on formed stools for the recovery of cysts which may be too few to be present in a single direct fecal film. A single iodine-stained film is employed for this concentrate.

2. *Types of specimens to be examined.* For routine examination films are prepared from the naturally passed stool. First of all, the stool should be carefully scrutinized macroscopically. If it is unformed, and particularly if it contains elements of blood and mucus, which are more likely to harbor trophozoites than is the fecal portion, examination should be made within a few minutes after the specimen has been evacuated, to prevent loss of motility of the amebas or their death and disintegration. Formed stools may be kept for a day or two in sterile containers in the ice-box without jeopardizing the diagnostic character of *E. histolytica* cysts, provided overgrowth of fungi does not occur. Formalin-fixed material is decidedly inferior to fresh stools for observing the critical nuclear characters of *E. histolytica*, but feces thoroughly comminuted in bulk with an equal amount of iodine stain (saturated iodine in 1 per cent potassium iodide), then tightly stoppered, are quite satisfactory. Moreover, the poly-vinyl-alcohol (PVA) fixation technic of the Communicable Disease Center, Atlanta may be employed for preparation of fecal films for later staining and examination, with assurance that the diagnostic criteria will usually be well preserved.

Three or four stools, obtained one every third or fourth day, should be examined before there is any real assurance that negative specimens constitute a negative diagnosis for the patient.

If passed stools are consistently negative and a suspicion of amebiasis remains, or if early diagnosis is urgent, saline-purged or enema specimens may be requested. Saline purgation evacuates material from the cecal area, including tissue debris, and *E. histolytica*, if present, is typically in the trophozoite stage. Sediment from the liquid specimen, usually passed after the formed feces have been evacuated, is most likely to yield positive results. Sodium sulfate or buffered phospho-soda is preferred to magnesium sulfate, since the former two salts appear to provide trophozoites in a more normal state of activity. Magnesia, bismuth, or oils should not be employed as purgatives, because the fine particles of the former two and minute oil droplets of the latter, as seen under the microscope, make diagnosis difficult and at times impossible. In case purgation is contraindicated a high physiologic saline enema may be employed. Sediment from this specimen should be examined as in the case of purged specimens.

Another potential source of *E. histolytica* is from proctoscopic scrapings, aspiration or punch biopsy. If infection has been contracted only a short time previously, the chances favor cecal amebiasis without sigmoido-rectal lesions, hence proctoscopic material is likely to be negative; but if the patient has chronic amebiasis proctoscopy may reveal typical or suspicious lesions from which samples may be obtained for immediate laboratory examination. Scrupulous care and discrimination are required in this type of examination, since mucosal cells, leukocytes, lymphoid cells or macrophages may be mistaken for quiescent amebic trophozoites, precysts, or even cysts. If typical *E. histolytica* motility is not demonstrated, it is advisable to return a tentative negative diagnosis. Probably more serious errors have resulted from false positive diagnosis based on proctoscopic samplings than from all other types of specimens submitted for laboratory diagnosis of *E. histolytica*.

In suspected extra-intestinal amebiasis the laboratory is frequently requested to examine aspirates, especially from hepatic and pulmonary abscesses or from the pleural cavity, rarely from the vagina. In all of these locations only the trophozoite stage is to be found. In amebic abscess of the liver it is not usual to recover active amebas from the necrotic material aspirated from the abscess cavity; only when the trochar probes the intact margin of the cavity is there definite probability that the amebas will be obtained in a motile, diagnosable state.

Additional sources of material for laboratory diagnosis of *E. histolytica* are from biopsy during surgical exploration and from necropsy. Scrapings or aspirates of suspected lesions should first be made for immediate wet-film examination, then representative tissues should be fixed in formalin (or preferably in Zenker's fluid), followed by sectioning and hematoxylin staining, in preparation for microscopic examination. If necropsy is more than four-hours old, autolysis may be moderately advanced, and the amebas in the tissues will usually have lost their diagnostic integrity. Under these circumstances superstaining with Best's carmine will make them stand out clearly against the tissue background.

Present-day culture methods are not recommended for routine procedure in the diagnosis of amebiasis, for the following reasons. If the stool is formed, only cysts will be present, and frequently excystation does not take place in the culture tube. Secondly, different strains of *E. histolytica* have different growth requirements and therefore require testing in several culture media. In the third place, culturing of large numbers of specimens for amebas is a laborious process. Finally, in culture it is at times difficult, if not impossible, to differentiate the trophozoites of *E. histolytica* from those of *E. coli*. However, cultivation at times may provide positive evidence of *E. histolytica* when fecal examination, saline purgation, enemas and proctoscopy have been negative. This is particularly true if the specimen submitted for examination is unformed (diarrheic, purged or aspirated). In case cultivation of *E. histolytica* in the test tube is attempted, the addition of a few hundred units of penicillin and streptomycin per tube will prevent overgrowth of fecal bacteria and thus improve the chances of finding the ameba.

Complement fixation is potentially a valuable diagnostic aid in amebiasis,

particularly following administration of antiamebic drugs, also in amebic hepatitis, in which demonstration of the amebas is usually not feasible. Nevertheless, it is not likely that serologic technics will supplant routine microscopic examination of specimens for the diagnosis of infection with *E. histolytica*.

3. *The Laboratory Diagnostician.* For dependable laboratory diagnosis of amebiasis the technician must be competently trained in this special field, must have experience, and must have diagnostic skill and sound judgment. Although typical cysts of *E. histolytica* can be readily recognized and differentiated from cysts of the other amebas and flagellates, as well as from *Blastocystis hominis*, more frequently the diagnostic characters of the ameba are less distinctive. The karyosome may appear to be eccentric, larger and more opaque than in the textbook picture, or the peripheral nuclear chromatin may be relatively coarse, suggesting *E. coli* rather than *E. histolytica*. Occasionally the mature *E. histolytica* cyst may have eight nuclei like *E. coli*. Moreover, there may be dense glycogen masses or numerous chromatoidals which completely mask the nuclei or obscure their distinguishing features. Likewise, tissue cells at times mimic amebas. An iodine-stained (or hematoxylin-stained) preparation may provide a picture in which the nuclear structure of a neutrophilic leukocyte or even a *Chilomastix* cyst may superficially resemble *E. histolytica*, so that the inexperienced diagnostician makes a diagnosis of *E. histolytica*. These examples demonstrate the need for well-trained technicians for this task.

Schools of laboratory technology have not provided dependable diagnosticians for amebiasis and other intestinal protozoan infections, even though they have turned out graduates who can do routine serological work fairly satisfactorily. Until relatively recently there were only a few persons in the United States who were regarded as experts in the diagnosis of *E. histolytica*, and even today experts at times differ from one another in the interpretation of the more difficult diagnostic specimens.

During the past few years the Laboratory Section of the Communicable Disease Center in Atlanta has provided training courses for directors and technical assistants of public health laboratories throughout the country, including intensive training in the diagnosis of intestinal protozoa. Follow-up tests on "unknowns" sent to the respective laboratories to test their proficiency have demonstrated that the training has been effective in the identification of *E. histolytica*. For the first time there are now a considerable number of individuals in state, county and city laboratories, who are competent to diagnose *E. histolytica*. Thus far, similar training has not been available for clinical pathologists and their technicians in private laboratories serving the majority of pay patients. This is a need which must be met if all classes of patients are to receive reliable diagnosis. Training courses should be instituted by state board of health laboratories to train these privately employed technologists.

DISCUSSION

The lack of uniformity in the diagnosis of amebiasis is apparent in both the clinical and epidemiologic fields. On the basis of the patient's history and physical examination, at times with the addition of X-ray and proctoscopy, but

without the benefit of microscopic examination of the stool, the physician may conclude that his patient has amebiasis and undertake anti-amebic treatment. This presumptive diagnosis may or may not be correct. Moreover, the overly "ameba-conscious" physician is apt to diagnose amebiasis in too many of his patients and then he may wonder why anti-amebic therapy does not consistently produce relief and clinical cure. Likewise, the unskilled laboratory technician may regard every undigested plant or animal cell in the stool as an ameba, or his microscopic technic may be so faulty that he fails to find *E. histolytica* which the experienced worker could readily find and identify.

It is difficult to decide whether a false positive diagnosis, arrived at solely on clinical grounds or after unsatisfactory laboratory examination, is more damaging than a missed positive diagnosis. However, both types of inaccuracy can be corrected if all suspected cases of amebiasis are given the benefit of laboratory examination by a properly trained medical technologist, whose findings are checked by a clinical pathologist equally skilled in the recognition of the diagnostic characters of *E. histolytica*.

Surveys to determine the prevalence of amebiasis in different communities and different population groups are difficult to compare with one another because of difference in the competence of the surveyor, in the collection of stool specimens, and in the thoroughness with which the examinations were made. In the same community and even in the same class of the population results have differed several-fold due to inequalities of sampling or of diagnostic ability. It is evident that the prevalence of amebiasis in a community can be accurately assessed as a public health problem only when it is based on dependable diagnosis.

Emphasis in present-day methods for the diagnosis of amebiasis has been placed on the importance of the microscopic recognition and differentiation of the etiologic agent, *E. histolytica*. Lest the reader conclude that amebiasis is exclusively a laboratory problem, it must be stated clearly that the physician in charge of the patient is professionally responsible for the interpretation of the laboratory findings. If a positive dependable laboratory report of *E. histolytica* is returned, then it is necessary to determine in the light of the clinical picture if the findings are of primary, secondary or incidental clinical importance. This the physician must decide and on this decision depends the management of his patient. If the laboratory diagnosis is negative on the basis of a single stool examination, and a clinical suspicion of amebiasis still exists, other stool specimens should be examined, supplemented by saline-purged or enema specimens. In cases of suspected amebic hepatitis aspiration may be contraindicated or the aspirated material may be negative for amebas. A positive stool may provide considerable support for presumptive diagnosis of amebic invasion of the liver but it is not absolute proof that the hepatic lesion is of amebic origin. Thus, it is evident that the laboratory is an essential supplement to the clinical examination and evaluation, but in no sense does it relieve the physician of his responsibility.

It is hoped that this brief presentation of the modern criteria for the diagnosis of amebiasis will serve some useful purpose, to the end that competent diagnosis of this infection will come to be regarded as essential and will become more general in the clinical laboratory. This, in turn, will engender a better understanding of the interdependence of the clinical and laboratory findings in amebiasis. The writer feels certain that these views coincide with the experience and judgment of the late Colonel Charles Franklin Craig, to whose memory this brief paper is dedicated.

CONCEPTS AND MISCONCEPTIONS IN AMEBIASIS

JOSEPH S. D'ANTONI

Tulane University of Louisiana School of Medicine, New Orleans, Louisiana

The late Colonel Charles Franklin Craig was an authority in many aspects of tropical medicine. It is doubtful that any other physician in the world knew more about amebiasis. He developed this interest during his tour of duty in Manila in 1906–1907 when he was serving with the Army Board for the Study of Tropical Diseases, and no matter how many other things occupied him during the remainder of his long and useful life, amebiasis remained his chief interest. As his associate and co-author Dr. Ernest Carroll Faust (1) wrote in his memoir of him,

> "In the hospital laboratory, at the autopsy table, and in the classroom he awakened American physicians to the dangers of amebiasis."

As his pupil and later as his associate in the Department of Tropical Medicine of the Tulane University of Louisiana School of Medicine, I can testify to the zeal Colonel Craig brought to that self-imposed task. My own compelling interest in amebiasis was first aroused by him. In the course of preparing this paper I looked over the correspondence which I had had with him after his retirement, the last of it shortly before his death in 1950, and much of it on this subject. What he would think of all the views I am expressing in this paper I cannot say, but with many of them I know that he was in hearty agreement.

In 1949 I sent Colonel Craig a paper on the public health and insurance aspects of amebiasis (3) in which I had recorded my decided convictions about these phases of the disease. I began with the following statement:

> "At the present time amebiasis occupies the anomalous position of being underestimated as to incidence, overestimated as to lethal possibilities, and largely mismanaged from the standpoint of public health."

Under date of December 12, 1949, Colonel Craig replied:

> "I wonder if you feel, as regards amebiasis, like 'a voice crying in the wilderness' so far as visible results are concerned. Well, keep on crying, and perhaps the results will come along without our knowing it."

Those of us who were privileged to study under, and work with, Colonel Craig, and who shared his own emphatic views about amebiasis, its unrealized prevalence, its unrecognized symptoms, and the enormous morbidity for which it is responsible, would stultify ourselves and show little regard for what he taught us if we did not "keep on crying". The task remains to be accomplished. As Doctor Faust (4) said in November 1950, in his presidential address before the American Academy of Tropical Medicine,

> ". . . public health and military authorities have regarded amebiasis as an unwanted stepchild, even when epidemics of the disease have occurred on their own doorsteps. Possibly they shun it because they . . . lack the courage to face the problem today."

It is a problem which must ultimately be faced. The organism which causes amebiasis was identified in 1875. It might reasonably be expected that over the

ensuing three-quarters of a century physicians would have come to some agreement about the disease. They have not. Two years ago the American Society of Tropical Medicine and the American Academy of Tropical Medicine set up a joint committee on amebiasis with the primary purpose of encouraging uniformity of concepts among those working in this field. The mere statement of the primary task carries its own unmistakable implications. The second objective of the committee, when the primary objective has been achieved, is the education of physicians, and then of lay persons, concerning amebiasis. As the chairman of this committee, I am afraid that I must assume a large part of the blame for its lack of progress. On the other hand, the real reason we have accomplished almost nothing is that we have not yet achieved any uniformity in our concepts of the disease. Unless and until we do, we obviously cannot undertake the education of physicians who do not specialize in tropical medicine, and certainly we cannot undertake to educate the public about a condition about which we ourselves are so divided. I still believe, however, that a committee with such objectives as these is our best hope. When one remembers what was accomplished by the committees which worked so effectively during World War II under the Office of Scientific Research and Development, notably in the control of malaria and typhus fever, we have no reason for complete discouragement.

One explanation for the existing confusion in concepts of amebiasis can be traced to the former unfortunate nomenclature. Since 1933, largely I believe owing to the efforts of Colonel Craig, Doctor Faust, and a few of their associates, the editors of the *Quarterly Cumulative Index Medicus* have ceased to index this disease as amebic dysentery and now index it, very properly, as amebiasis. Yet many physicians (and much of the public along with them) continue to call amebiasis, amebic dysentery, and continue to overlook it when it does not occur in that manifestation, as in the United States it usually does not. Amebic liver abscess, incidentally, continues to be indexed under "liver, amebic abscess of," with no reference at all to amebiasis, which means that its role as a complication of that disease is blurred and distorted.

INCIDENCE AND MORBIDITY

When Colonel Craig (1927), first stated, that from 5 to 10 per cent of the population of the United States suffered from amebiasis, his remarks were greeted with a skepticism, a good deal of which still persists. I know that he never swerved from that opinion and that he came to believe, instead, that he had underestimated the prevalence of the disease. In his text on amebiasis (6), in fact, he cited with approval Faust's surmise that if several examinations were made by improved technics in all patients suspected of harboring amebic infection, the general incidence in the United States might well prove to be 20 per cent.

As a matter of fact, until reliable surveys are made in many parts of the country by efficient technics, no authoritative statements can be made about the real incidence of amebiasis. A number of facts suggest, however, that it is very much higher than many physicians believe it to be. For one thing, there are a good many reasons for questioning the general level of accuracy of diagnosis. As

Colonel Craig repeatedly emphasized, the competency of the examiner determines the number of positive diagnoses made. I have often heard him say that for a physician or a technician to be able to diagnose 90 per cent of the amebic infections which come under his observation, he must be trained for a period of two years under qualified supervision and should, preferably, examine thirty or forty stools a day during that period. How many physicians and how many technicians, outside of special laboratories of tropical medicine, come anywhere near meeting those criteria?

Colonel Craig (6), although he repeatedly emphasized it, was not the first to point out that examination of a single normally passed stool will reveal only a small proportion of positive cases, while a negative result has no value at all. As long ago as 1917 Dobell (7) made the same point; not more than a third of the infections in any given area surveyed, he said, will be revealed by this method. That is a very generous estimate. Amebiasis constitutes a large part of my practice, and not a week passes that special technics do not reveal in my own laboratory two to four positive diagnoses in patients who had previously been told, after the examination of from one to several normally passed stools, that they did not have amebiasis.

Other points of diagnostic importance include the methods by which specimens are collected, the amount of time spent in the examination, and the technics utilized, that is, plain smears, concentration technics, and culture technics. No one but Colonel Craig himself ever had much success with complement-fixation tests, chiefly I believe because very few workers ever took the trouble to follow the precise technic which he described.

If we know little of the real prevalence of amebiasis, we know still less of the ill health which it causes. There are no spontaneous cures, and most students of the disease believe, as Colonel Craig did, that all untreated amebiasis will ultimately give rise to symptoms. In a very large number of cases, however, the symptoms to which amebic infection gives rise are attributed to other causes.

I should like very much to see some well organized clinic, on whose staff is a physician well trained in tropical medicine, investigate a thousand consecutive patients from the standpoint of possible amebiasis, regardless of the symptoms for which they have sought medical advice. That means, of course, that there would be an intensive search for *Endamoeba histolytica*, by efficient technics. I suspect that the incidence of infection would prove even higher than the 20 per cent which Faust postulated for the general population and that in perhaps half of all the infected cases amebiasis would prove to be the only disease present. So far as I know, no survey of this sort has ever been carried out. It is an enormous task, I grant, but only after studying the results of a representative number of such investigations shall we be able to speak authoritatively about amebiasis in terms of the clinical illness which it produces.

EPIDEMIOLOGY

The epidemiology of amebiasis is another phase of the disease which remains to be clarified. On the surface, it seems an anomaly that it should be widely prevalent in a country where, at least in urban areas, outside privies are almost

unknown, where night soil is scarcely ever used for fertilization, where screening is fairly general in the areas in which it is needed, and where sand filtration of water and sewage disposal are usually adequate, even in small towns. In short, we are faced with the curious situation that in the best sanitated country in the world, the incidence of an intestinal disease is at least stationary and is probably increasing.

Colonel Craig himself leaned to the theory that in the United States food-handlers are probably responsible for most cases of amebiasis. I have heard him say on many occasions that while they might not furnish the whole answer, he would stick with them until a more reasonable theory should be advanced. If one accepts this hypothesis at all, then the point made by Andrews (8) seems well taken, that since the mother is responsible for the preparation of the family food, attention to her status might do much to reduce the familial spread of the disease. Be this as it may, the studies which I have been carrying out since 1947 with Doctor Maud Loeber indicate the possibilities of transmission of the disease from parent to child.

To date I have observed 550 cases of amebiasis, all proved by laboratory methods, in children ranging in age from 1 month to 12 years. In 425 of these children the laboratory investigation was undertaken because the symptoms suggested that this disease might be present. In the remaining 125 cases symptoms were vague and the laboratory examinations were carried out because the children were members of families in which amebiasis had already been diagnosed. In something over 80 per cent of the 550 cases the correctness of the diagnosis was additionally confirmed by the marked clinical improvement which followed effective amebicidal therapy.

The most significant part of this investigation of amebiasis in children is the relationship which we have been able to trace between parents and children in the transmission of the disease. It is unfortunate that more parents could not be examined, but cooperation in this respect is never too easy to obtain. The results were striking, however, in the cases in which examinations were made: *E. histolytica* was found in 71 of the 76 mothers, and in 50 of the 54 fathers who submitted purgative specimens. Moreover, the infection was so heavy in about 80 per cent of the cases that the parasites were found in the first specimen examined, which is by no means the usual diagnostic experience. We have come to believe, in fact, that the parent who infects the child must be a heavy cyst-passer.

While I do not yet have sufficient data to prove it, I am gradually coming to believe that many of the amebic infections encountered in adults are probably contracted in the first year or two of life, from parents and nurses. I am aware of several studies elsewhere in Louisiana, as well as in other parts of the country, which in general confirm these observations, and I hope that as time passes, more students of amebiasis will interest themselves in the childhood phase of the disease. When I reported my observations to Colonel Craig, I was encouraged to have him write me (July 8, 1948):

> "This work adds a landmark to our concept of the incidence of the disease. In my own experience I have examined very few children, being in the Army and doing most of my work among adults, so that I certainly missed this

phase of the subject. I am in agreement with you that the infection in children is derived from members of the family in contact with the children. Your findings would certainly appear to prove this method of infection."

CLINICAL MANIFESTATIONS

Colonel Craig was one of the first students of amebiasis to call attention to the fact that amebic ulceration of the intestinal tract and amebic liver abscess can exist without dysenteric symptoms, or even a history of parasitic infection. I have already commented on the still distressingly general use of the former nomenclature of "amebic dysentery", and the diagnostic error to which it gives rise. In a practice limited for the past sixteen years to diseases of the colon, I would say that in this country not more than 5 per cent of the patients with amebiasis give a history of dysentery. Even intermittent bouts of diarrhea, which are far more common, are not present in more than 20 to 25 per cent of all cases. Furthermore, as Colonel Craig emphasized in so many of his writings, amebiasis can exist without any symptoms at all referable to the intestinal tract. Physicians who are not aware of these facts fail to suspect the possibility of amebiasis in four out of every five cases which come under their observation.

In this connection, one recalls the 700 patients with amebiasis whose 1,961 separate complaints Boyers (9) once took the trouble to tabulate. Appetite, digestion, bowel habit, respiratory and cardiovascular function, urinary function, sexual status, skin and mucocutaneous areas, the glandular system, memory and morale were all affected, but the symptoms referable to the gastrointestinal tract made up less than 30 per cent of the total number of complaints. Unless physicians learn the possible clinical manifestations of amebiasis, Boyers concluded, the problems which this disease presents in temperate and subtropical zones will continue to go unsolved.

Amebiasis, unfortunately, has no typical clinical picture, which is one reason why it is so difficult to present to both physicians and medical students. I am increasingly impressed, however, with the frequency with which certain special symptoms appear, though I hasten to add that most of them also appear in many other patients who do not have amebiasis:

1. Fatigue, which is characteristic, takes two forms. Some patients awaken fatigued, but by evening have lost much of their morning weariness. Others become abnormally fatigued as the day progresses, especially if they are called upon for any unusual exertion.

2. Headaches, which are very frequent, are of two types, a recurrent headache and a headache which lasts for three to ten days at a time. Either variety may be regarded as migraine. Most patients complain of frontal headaches. Others complain of pain in the base of the skull and in the trapezius muscle. I have had patients with the latter type of discomfort tell me that they have knots in the muscles of the neck.

3. Nervousness may be so pronounced that the patient is treated for psychosomatic disease, with no recognition at all of the infectious origin of the trouble. It is a rare patient, in fact, who has not at some time or other been described as neurotic.

4. Many patients, who are frequently unaware of it until they are requested to take their temperature regularly, have a low-grade afternoon elevation, which seldom exceeds 100°F.

5. A fairly large number of patients have symptoms suggestive of disease of the hepatobiliary system, including epigastric fullness after meals, epigastric distress, belching, nausea, and sometimes vomiting. Hepatomegaly may or may not be present. It is observed in not more than 10 to 15 per cent of all adult subjects, though it is very frequent in young children.

It must be emphasized again that the symptoms and signs just described do not represent what we ordinarily understand by the term amebic hepatitis, which results from the invasion of the liver by *E. histolytica*. My own opinion, which I admit is still based only on clinical evidence, is that they represent a toxemia which originates in toxins produced in the infected colon. I hope shortly to be able to present investigative work which will substantiate the clinical impression.

A number of other symptoms, which are not usually regarded as part of the disease, appear frequently in patients with amebiasis and must be in some way related to the infection, for they are greatly improved, or disappear entirely, after successful amebicidal treatment. Perhaps it might be well to devote more attention to them. They include a group of gynecologic complaints observed in young girls as well as in older women who have no gynecologic findings to explain them, such as menorrhagia, metrorrhagia, dysmenorrhea, and menopausal symptoms; a urinary syndrome in women consisting of recurrent cystitis, urethritis with demonstrable urethral stricture, and diminished bladder capacity; pruritus ani; chronic dermatologic conditions; rectal bleeding; symptoms clinically identical with those of fibrositis; dizziness; chronic low-grade anemia; and symptoms suggestive of chronic migratory polyarthritis.

DIAGNOSTIC CONSIDERATIONS

The advantages and disadvantages of the two fundamental technics used in the laboratory diagnosis of amebiasis, namely, hematoxylin (fixed) and iodine (wet) preparations, need not be entered into again at this time. There is a great gulf fixed between the physicians who use the one and those who use the other. Very few men are able or willing to use both methods, and the result is that a positive diagnosis made by a physician using one technic often cannot be substantiated in the same specimen by a physician who uses the other technic.

I should like to record at this time a point of diagnostic importance in connection with amebiasis in children, that examination of material aspirated from the bowel wall is far and away the most efficient method of diagnosis. In only 25 of the 550 children with amebiasis whom I have observed did this type of specimen prove negative when purgative stools, enema specimens, or both were positive. In the whole series, enema specimens were positive in 93 cases (17 per cent of the total number), purgative specimens in 134 cases (24 per cent), and aspirated material in 471 cases (85 per cent). The diagnosis was made on the first sigmoidoscopic examination in 428 cases (78 per cent), on the second in 63

cases (11 per cent) and on the third in 34 cases (6 per cent). It is customary to use all three types of specimens, however, for the sake of completeness, since nonpathogenic protozoa and helminths are picked up more readily in purgative and enema specimens than in aspirated material.

Some of the disagreements over the laboratory diagnosis of amebiasis would disappear if it were remembered that the disease in the United States does not take the same form that it does in tropical countries. In the tropics the output of parasites is usually very heavy; in the United States it usually is not. A native of the tropics, who lives in a warm or hot climate, who is malnourished by American standards, and who often harbors more than one tropical disease, presents a sharp contrast to an American, brought up in a temperate climate, who is well-nourished, and who is sulfa-, antibiotic- and vitamin-conscious. Diagnosis in the former patient would probably present few difficulties. In the latter, the examination would take more time and would require the use of more refined technics.

Physicians who have worked in the tropics, in addition to insisting that numerous parasites must be present for diagnosis, also insist that parasites must be morphologically typical in this country, as they are in the tropics. Some even declare that physicians whose experience is limited to the United States sometimes identify leukocytes as *E. histolytica* organisms. I can easily refute that criticism from my own experience: it takes me from 50 to 70 per cent longer to make a diagnosis of amebiasis in cold weather, because parasites are less frequent in winter than in summer. Leukocytes have no seasonal variation in incidence.

Another criticism sometimes heard is that the diagnosis of amebiasis is not justified if active trophozoites are not found in the stools. If we accept the concept, which Faust and others have established, that amebiasis is primarily a disease of the cecum, then trophozoites will be found in the stools only when diarrhea is a part of the disease, as in the United States it frequently is not.

Finally, the criticism might be mentioned that the diagnosis of amebiasis is not reliable in any laboratory which does not report an equally large, or even larger, incidence of nonpathogenic protozoa. This might be a sound argument in the tropics, or in the United States in patients of low economic status, living in poor sanitary surroundings where opportunities for reinfection are frequent. Ordinarily, however, although untreated *E. histolytica* infections persist over periods of years in the United States, infections with nonpathogenic protozoa do not.

THERAPY

In the Tulane University School of Medicine, one of the requirements in the postgraduate course in tropical medicine is the presentation of an acceptable thesis. My own contributions to the list offered for selection to the students always includes "The Criteria of Cure in Amebiasis". The subject rarely goes unselected. It sounds interesting, and it looks easy, which it is not. Almost invariably the physician who has selected it comes to me, after he has worked on

the thesis for two or three weeks, to tell me that the task is impossible, that many of the data essential to evaluate the efficiency of special drugs are not supplied in the literature, and that no two writers seem to have the same criteria of cure. I then explain that the wording of the title was deliberate; we set up criteria which we would jointly consider ideal, and the student returns to the literature with a new point of view. The thesis which results is usually enlightening.

We shall never develop efficient drugs in amebiasis unless we agree on what basis their efficiency is to be evaluated. A consideration, for instance, that is generally overlooked is the patient himself, who ought to be relieved of his symptoms before he is pronounced free of his disease. One of the excellent things that happened medically in World War II was that so many of the men who were called upon to treat amebiasis were well-trained clinicians, whose first criterion of cure was the amelioration of all symptoms, not merely a negative report from the laboratory.

Another point to be agreed upon is the length of time that should elapse after medication has been completed before stools are examined for possible drug failure. There is no set time. It depends entirely upon the drug employed. My own experience with Milibis and chloroquine indicates that a minimum of four to six weeks must elapse after therapy before material aspirated at sigmoidoscopy again becomes positive. Purgative specimens do not again become positive for three to six months.

The recurrence of symptoms in cases in which parasites cannot be found on repeated re-examinations is admittedly puzzling. The temptation is great to settle the matter by calling the patient neurotic, but it is one that should be sternly resisted. It is much wiser to repeat the course of therapy. If symptoms are again relieved, then the physician should look into the efficiency of his own laboratory technics. This is a fair enough criticism, for, with the exception of chloroquine, which is also useful in malaria, none of the most commonly used amebicidal drugs will cure any disease except amebiasis.

As a matter of fact, there is no correlation between the output of parasites and the severity of clinical symptoms. I have more than once treated patients who were incapacitated by their symptoms but who required repeated examinations before even occasional parasites were found by any technic. It is also possible for the output of parasites to be materially reduced after therapy although at the same time the patient presents severer symptoms than he presented originally, or even presents new symptoms.

An evaluation of the drugs used in amebiasis at the present time is not part of my purpose, but I should like to conclude these remarks with some comments based upon my own clinical experience. Diiodohydroxyquinoline (Diodoquin) began to lose its efficiency in my hands about 1946. Emetine, as Colonel Craig first noted in 1917, and as he repeatedly stressed thereafter, is very useful in controlling acute dysentery and diarrhea but is not helpful in the actual eradication of amebic infection. In August, 1949, he wrote me that he had received conflicting reports about chloroquine, and most of them were bad. In

my experience, it has been, like emetine, a useful drug in amebic hepatitis and amebic liver abscess, but not in intestinal amebiasis. The promise of aureomycin has not been fulfilled, and my own experience with terramycin suggests that the same fate is likely to befall it. In March 1950 Colonel Craig wrote me that he would not himself advocate the use of aureomycin, which is my own feeling about it. At this time Milibis seems the most efficient amebicide available, though it must be used in larger dosages than the manufacturer recommends.

SUMMARY

Amebiasis at the present time is a confused, incompletely understood, and much misunderstood, disease. The real prevalence is unknown. The epidemiology remains to be clarified. The clinical manifestations are typically atypical, and are frequently not referable to the intestinal tract. The diagnosis of the disease, even though it is entirely a laboratory matter, is sometimes in dispute. The criteria of cure differ from physician to physician, and no entirely satisfactory amebicidal drug is presently available. The pupils and associates of the late Colonel Charles Franklin Craig have wide avenues of opportunity open to them in the investigation of every phase of amebiasis, but much of the confusion which now beclouds the disease might be cleared away if more attention were paid to the principles which he enunciated concerning it.

REFERENCES

In future issues of this Journal, references will be cited by date and not by number.

1. Faust, Ernest C., Charles Franklin Craig (1872-1950). An appreciation, *Am. J. Trop. Med.*, **31:** 267–269, 1951.
2. Craig, Charles F., Personal Communications.
3. D'Antoni, Joseph S., Public health and insurance aspects of amebiasis. A note on certain erroneous concepts, *New Orleans M. & S. J.* **102:** 55–60, 1949.
4. Faust, Ernest C., Seeing ourselves, *Am. J. Trop. Med.* **31:** 153–157, 1951.
5. Craig, Charles F., The symptomatology of infection with *Endamoeba histolytica* in carriers, *J. A. M. A.* **88:** 19–21, 1927.
6. Craig, Charles F., The Etiology, Diagnosis and Treatment of Amebiasis. Baltimore, Williams & Wilkins Company, 1944.
7. Dobell, cited by Craig (6).
8. Andrews, Justin, Amebic dysentery (Letters to the Editor), *J. A. M. A.* **101:** 821, 1933.
9. Boyers, L. M., Human amebiasis; a review of 700 cases expressing 1961 complaints, *Calif. & West. Med.* **39:** 397–401, 1933.

THE CULTURAL REQUIREMENTS OF *ENDAMOEBA COLI* AND THE COMPARATIVE EFFECTS OF DRYING ON THE CYSTS OF *E. COLI* AND *E. HISTOLYTICA*

LUCY V. REARDON, ELISABETH VERDER[1], AND CHARLES W. REES

Laboratory of Tropical Diseases and Laboratory of Infectious Diseases, National Microbiological Institute, National Institutes of Health, Bethesda 14, Maryland

The studies herein reported were begun early in the year 1950 with the objective of ascertaining methods of cultivating *Endamoeba coli* with known species of microbial associates throughout all phases of the life cycle. Previous reports from this laboratory have indicated that excystation of *E. coli* may be induced under conditions similar to those required for *E. histolytica* (1, 2). Despite this similarity between the two species, metacystic *E. coli* failed to produce cultures of trophic amebas even when organism *t* or *Trypanosoma cruzi* was added, whereas with either of these associates *E. histolytica* has been readily cultivated (3, 4). In a preliminary abstract (5) in which some of the early difficulties in obtaining a culture of *E. coli* were indicated, the establishment of one culture from a concentration of cysts was reported. Continued work on this culture has provided some data of the present paper. Other data to be reported here were the outgrowth of the surprising development of a culture, following transfer of a concentration of cysts of *E. coli* from a dish in which the cysts had dried, to a tube of whole egg medium. This result led to studies on the comparative effects of drying on the cysts of both species of *Endamoeba*.

EXPERIMENTAL DATA

The cultures of *E. coli* for these studies were obtained from cysts in fecal specimens of freshly passed stools or in stools that had been stored up to 15 days at 5°C. The cysts were concentrated by the zinc sulfate flotation method described by Tobie and associates (6) excepting that the floated cysts were collected from narrow necked tubes of a design described by Snyder and Meleney (7). The tubes used by us were constructed from 18 x 150 mm. test tubes by cutting off and discarding approximately 75 mm. from the open end and sealing on an equal length of 9 mm. glass tubing. Cyst suspensions collected from the necks of two tubes were washed several times in N/20 HCl and through several changes of 0.85 per cent NaCl, or in some cases through the NaCl solution only. The sediment from the final washing was resuspended in the saline and transferred to a watch glass from 50 to 100 mm. in diameter and allowed to settle for approximately 30 minutes. The container was then inverted with the result that most of the fecal debris was eliminated, but many of the cysts were found in the film of saline remaining on the watch glass. The photomicrograph (Figure 1) shows a typical watch glass concentrate. For comparative experiments on the effects of drying, the cysts of *E. histolytica* were also obtained from stools by

[1] Laboratory of Infectious Diseases.

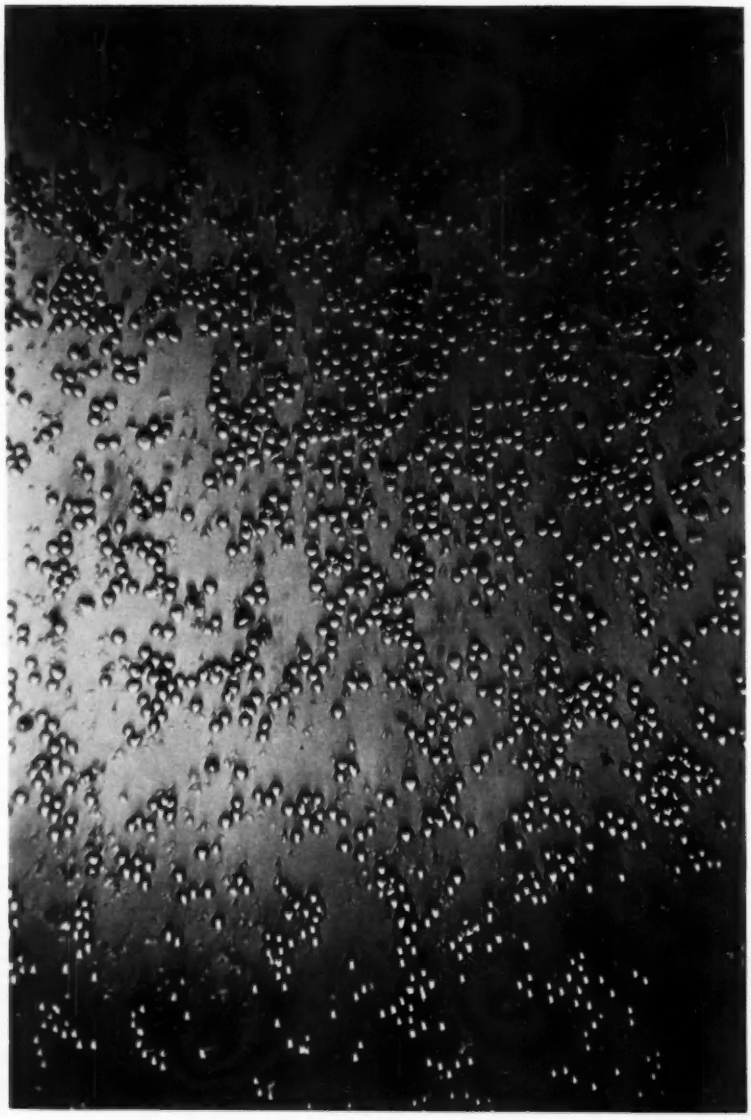


FIG. 1. Photomicrograph of a concentrate of *Endamoeba coli* cysts.

the same methods. Except where otherwise indicated riced whole egg medium was used for the experiments on cultivation.

In the first culture of *E. coli* mentioned above the published report indicated that organism *t* and a species of *Geotrichium* were present (5). However, on serial transfer a diphtheroid and *Bacillus subtilis* were also identified. The question whether these additional species were present from the beginning or were introduced through contamination cannot be answered, but at any rate, the growth of *E. coli* was scanty until evidence of the presence of *B. subtilis* was obtained. In order to ascertain which species of associated organisms are necessary to support good growth of *E. coli*, efforts were made to simplify the flora of the culture. The first attempts, using antibiotics, penicillin and streptomycin, were unsuccessful. A method designated as "coverslip transfer" was then used with the result that the diphtheroid and *Geotrichium* were eliminated, and a culture of *E. coli* was obtained with organism *t* and *B. subtilis*. The coverslip technique was conducted as follows: A strip of sterile coverglass was inserted into an ameba culture containing the four species of associates, left during 72 hours of incubation, then withdrawn, passed through four tubes of sterile medium, and deposited in a fifth tube. In experiments comprising from 10 to 20 series of coverslip transfers, amebas grew in most of the tubes through which a coverslip had passed and in some of the final tubes, in one of which the above-mentioned culture of *E. coli* with organism *t* and *B. subtilis* developed. These two species of bacteria were therefore seeded into cultures with microisolated cysts and good growth of a number of strains of *E. coli* has thus been produced.

In one of these strains, occasional cysts were seen and intensive studies were conducted in attempts to obtain greater numbers of cysts. Success was finally achieved with the use of the well-known method of Dobell (8) for obtaining cysts from cultures of *E. histolytica*. By this method the amebas are grown without starch and then transferred to medium with starch, in which encystation occurs. However, *E. coli* could not be grown without starch when organism *t* and *B. subtilis* were the only associates. On the other hand, when the flora of the NIH strain of NRS *E. histolytica* was separated from the amebas by subculture in nutrient broth and added to the culture of *E. coli*-organism *t*-*B. subtilis*, some starch-free growth of this ameba occurred, with encystation following transfer to riced medium. Additional studies have now shown that starch-free growth and encystation of *E. coli* may be obtained in the presence of only three species of bacteria, organism *t*, *B. subtilis*, and *Streptococcus zymogenes*-NIH 1308 (9). This information was obtained from experiments with a total of 16 species of bacteria which were added, one species in each experiment, to cultures of *E. coli*-organism *t*-*B. subtilis*. Culture-cysts have been found to produce cultures of amebas when microisolated into medium by the same method described for stool-cysts.

The procedure for the drying experiments differed from other cultural studies in that the preparations were dried in small drops of 0.85 per cent NaCl on sterile strips of coverglass before introduction into the medium rather than by transfer directly from the micropipette. Nondried controls were used in all

cases. Drying was carried out in the air of the laboratory the temperature of which was at or near 80°F. and the relative humidity, as determined by wet and dry bulb readings, ranged from 25 to 30 per cent. With one exception viability measurements were made on the cysts used in the experiments. This was done by the introduction of bacteria-free cysts into sterile liver infusion agar medium in microtubes with 10 cysts per tube, and examination after overnight incubation to ascertain the percentage of cysts that hatched in 5 to 10 microcultures. This percentage constituted a viability index. Experiments, not reported here in detail, showed that high viability indices were correlated with high percentages of positive cultures of amebas.

TABLE 1

Comparative effects of drying on the culturability of cysts of E. coli and E. histolytica. Cysts on strips of coverglass were inserted into tubes of riced whole egg medium either immediately or from 10 to 30 minutes after the preparations were dried

The medium was seeded with indicated species of bacteria

EXP. NO.	SPECIES OF *Endamoeba*	SPECIES OF BACTERIA	SOURCE OF CYSTS	VIABILITY TEST	CYSTS PER TUBE	RESULTS			
						Dried cysts		Nondried cysts	
						Tubes seeded	Pos. for ameba	Tubes seeded	Pos. for ameba
				per cent	*no.*	*no.*	*no.*	*no.*	*no.*
1	*E. coli*	org. *t-B. subtilis*	stool 7	28	20	5	5	5	4
2	*E. coli*	org. *t-B. subtilis*	stool 7	28	25	10	10	10	7
3	*E. coli*	org. *t-B. subtilis*	culture	4	20	10	3	10	6
4	*E. histolytica*	organism *t*	stool 8	36	20	10	0	10	10
5	*E. histolytica*	organism *t*	stool 8	36	30	4	0	3	3
6	*E. histolytica*	organism *t*	stool 8	36	10	3	0	5	4
7	*E. histolytica*	organism *t*	stool 9	not read	20	5	0	5	1
8	*E. histolytica*	organism *t*	stool 9	not read	20	5	0	5	3
9	*E. histolytica*	organism *t*	stool 10	14	25	10	1*	10	7

* Only one trophozoite seen.

The cultural data on the comparative effects of drying on the two species of *Endamoeba* are summarized in Table 1. There was no evidence of damage to the cysts of *E. coli* due to drying whereas, with one exception, the cysts of *E. histolytica* appeared to have been irreversibly injured by such treatment. No culture of *E. histolytica* was established from dried cysts, but a trophozoite was found in examinations of one tube.

The effects of drying on the two species of *Endamoeba* were also studied microscopically, during drying, after restoration of 0.85 per cent NaCl, and after staining with iodine. The successive stages of drying, illustrated in figure 2, show indentation of the cyst wall until the cysts appeared as crescents and finally were indistinguishable in the salt crystals. Following restoration of the saline solution the cysts of *E. coli* regained their normal appearance, while those of *E. histolytica* showed some retraction of the protoplasm from the cyst wall.

In the iodine stain the nuclei of the *E. coli* cysts were distinct, whereas those of *E. histolytica* cysts were cloudy. These observations are in agreement with the cultural studies.

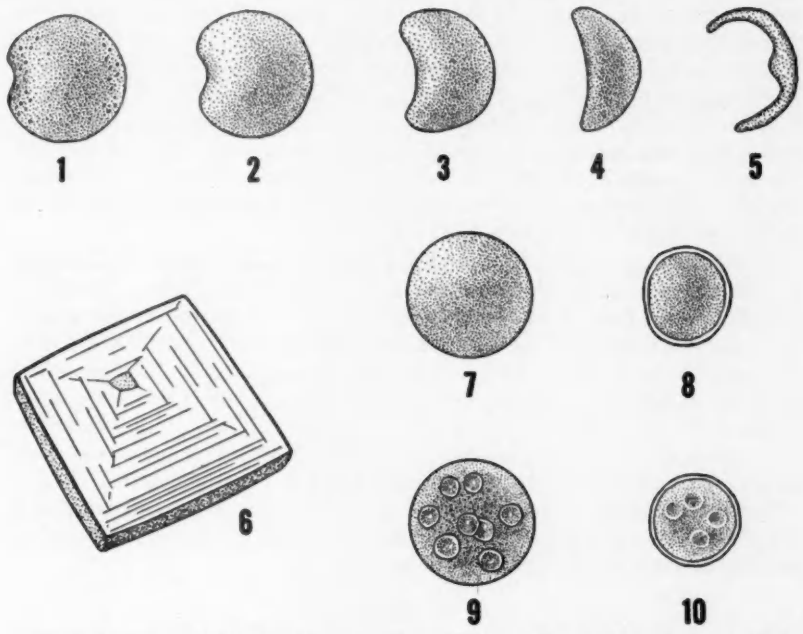


FIG. 2. Diagrammatic illustrations of cysts of *E. coli* and *E. histolytica*, during drying in 0.85 per cent NaCl, after restoration of the saline solution, and after staining with iodine. Drying occurred in air at 80°F. and 25 per cent relative humidity. 1 to 5—stages of drying; 6—salt crystal; 7 and 8—*E. coli* and *E. histolytica* respectively after restoration of saline solution; 9 and 10—after staining with iodine.

DISCUSSION

The principal contribution of the cultural data of the present paper lies in a demonstration, for the first time, of methods whereby *E. coli* may be cultivated from microisolated cysts with known microbial associates. The data on the comparative effects of drying on the cysts of *E. coli* and *E. histolytica* may be found useful in studies on the epidemiology of amebiasis and may indicate factors contributing to the greater prevalence of infections with the nonpathogenic species than with the pathogen. On the other hand, the results thus far obtained may be different from those that might occur under conditions of temperature and humidity other than those herein reported, or when drying may occur in fluids other than 0.85 per cent sodium chloride. Further studies along this line are indicated.

For many years *E. coli* has been known as a nonpathogenic parasite confined

within the lumen of the lower intestinal tract, and *E. histolytica* as a pathogen which may invade the intestinal mucosa and deeper layers and migrate therefrom to the liver and/or other organs. Data indicating differences between the cultural requirements of the two species are, therefore, not surprising, even though the cultural differences thus far shown are not marked. *E. coli* may require more factors supplied by bacteria than *E. histolytica*. A possible explanation is that *E. coli* may be a more strict anaerobe than *E. histolytica* and may thus require more complete consumption of the oxygen in the present instance by *B. subtilis*, than is effected by organism *t* alone. Comparative studies on the sites of infection in the host may lend some support to this hypothesis since, although both species have the same environment in the intestinal lumen, *E. histolytica* may have a greater tolerance for oxygen, permitting it to live in the tissues in contact with or in close proximity to the blood stream.

B. subtilis may contribute also to the growth of *E. coli* through its action on particles of ground rice. Observations on this material in cultures of *B. subtilis* without amebas indicated that the starch grains were liberated from protein envelopes in a manner similar to, if not identical with, the enzymatic reaction on rice particles by *E. histolytica* in culture with organism *t* (10). This bacterium unlike *B. subtilis* does not liberate the starch grains in the absence of amebas. Evidence for the presence or absence of an enzyme in *E. coli* acting similarly to the enzyme of *E. histolytica* has not been obtainable because *E. coli* has failed to grow in the absence of species of bacteria that act on the rice.

Previous accounts of the cultivation of *E. histolytica* with single species of microbial associates and the present account on *E. coli* all point to the need for investigating the metabolism of the associates.

SUMMARY

1. A modification of the zinc sulfate centrifugal flotation technique is described for concentrating cysts of *E. coli* and *E. histolytica* from stools and cultures in watch glasses with minimum amounts of foreign material.

2. Several strains of *E. coli* have been cultivated from cysts that were freed by microisolation from bacteria and transferred into riced whole egg medium that was seeded with organism *t* and *B. subtilis*.

3. Encystation of *E. coli in vitro* was obtained by the method of Dobell in cultures with an undetermined number of species of bacteria and also with only three species, organism *t*, *B. subtilis* and *Streptococcus zymogenes*.

4. On the basis of culturability and microscopic appearance the cysts of *E. coli* were not demonstrably affected by drying; cysts of *E. histolytica* were irreversibly damaged by drying.

REFERENCES

In future issues of this Journal, references will be cited by date and not by number.

1. Rees, C. W., Reardon, L. V., Jones, F. E., and Griffin, A. M.: Observations on the excystation of *Endamoeba histolytica*. *J. Parasitol.* **33**: 385, 1947.

2. Rees, C. W., Reardon, L. V., and Bartgis, I. L.: The excystation of *Entamoeba histolytica* without bacteria in microcultures. *Parasitology*. **40**: 338–342, 1950.

3. Rees, C. W., Bozicevich, J., Reardon, L. V., and Jones, F. E.: A preliminary note on the complement fixation test for amoebiasis with antigens prepared from *Endamoeba histolytica* grown with a single species of bacteria. *Am. J. Trop. Med.* **22:** 581–586, 1942.
4. Phillips, B. P.: Cultivation of *Endamoeba histolytica* with *Trypanosoma cruzi*. Science **111:** 8–9, 1950.
5. Rees, C. W.: Experiments on excystation and growth of *Endamoeba histolytica* and *Endamoeba coli*. *J. Parasitol.* **36:** 6(Sec. 2), 37, 1950.
6. Tobie, J. E., Reardon, L. V., Bozicevich, J., Shih, B-C., Mantel, N., and Thomas, E. H.: The efficiency of the zinc sulfate technic in the detection of intestinal protozoa by successive stool examinations. *Am. J. Trop. Med.* **31:** 11–111, 1951.
7. Snyder, T. L., and Meleney, H. E.: The excystation of *Endamoeba histolytica* in bacteriologically sterile media. *Am. J. Trop. Med.* **21:** 63–73, 1941.
8. Dobell, C.: Researches on the intestinal protozoa of monkeys and man. I. General introduction, and II. Description of the whole life-history of *Endamoeba histolytica* in cultures. *Parasitology* **20:** 357–412, 1928.
9. Evans, A. C. and Chinn, A. L.: The enterococci: With special reference to their association with human disease. *J. Bacteriol.* **54:** 495–512, 1947.
10. Reardon, L. V. and Bartgis, I. L.: The cultivation of the small race of *Endamoeba histolytica* with a single species of bacteria. *J. Parasitol.* **35:** 218, 1949.

EXPERIMENTAL *ENDAMOEBA HISTOLYTICA* INFECTIONS IN RABBITS WITH REFERENCE TO CHEMOTHERAPY

GEORGE W. LUTTERMOSER, W. T. HASKINS, NELL COLEMAN, AND J. R. JUMPER

National Institutes of Health, National Microbiological Institute, Laboratory of Tropical Diseases, Bethesda 14, Maryland

Experimental infections of *Endamoeba histolytica* have been established in several laboratory animals, many of which have been utilized in chemotherapeutic studies. Jones (1948) ably reviewed the history of the infection of dogs, cats, and monkeys with *E. histolytica* and stated that "such valuable amebicides as stovarsol, carbarsone, Vioform, and Diodoquin have resulted." Anderson *et al.* (1947, 1950) have further shown the activity of some thioarsenites and antibiotics against natural amebic infection in the monkey. The suitability of the dog as a test animal was studied critically by Thompson *et al.* (1949, 1950) who reported a satisfactory correlation between the response of canine and human amebiasis and stated that the dog should be particularly useful in the search for new chemotherapeutic agents. Clampit (1948) found that some cases of acute fulminating amebiasis produced experimentally in the kitten were cured by the use of Vioform, chiniofon, and carbarsone but none were cured by emetine. Jones (1946, 1947) demonstrated the successful infection of young rats by the intracecal inoculation of trophozoites of *E. histolytica* and measured the amebicidal activity of proven drugs against this infection. Dennis *et al.* (1949) freed the hamster of a natural parasite, *Endamoeba criceti*, by administration of Diodoquin, chiniofon, carbarsone, and by the use of p-N-glycolarsonilate (Milibis). It was demonstrated by Tobie (1949) that the rabbit can be infected by intracecal inoculation with trophozoites of *E. histolytica*. The lesions developing in this infection were similar to those seen in the acute ulcerative disease in man. Taylor *et al.* (1949, 1950) and Carrera and Faust (1949) used the same method to produce acute amebiasis in the guinea pig.

As part of a project to determine the mode of action of Diodoquin, Vioform, and chiniofon in amebiasis, the present study was undertaken first to see whether the results of Tobie were reproducible in a large series of animals, secondly to define further the characteristics of experimental amebiasis in the rabbit, and finally to study the response of the disease to therapy with these iodinated quinolines and other drugs. The physiological disposition of Diodoquin, Vioform, and chiniofon in the rabbit have been described previously as well as a method for determination of 8-quinolinol in urine (Haskins *et al.* 1950, 1951).

MATERIALS AND METHODS

The rabbits utilized were New Zealand giants about 6 to 12 weeks old. At least 7 days before inoculation, they were placed on a deficient diet (Tobie, 1949) consisting mostly of oats with some corn and bran. In a few tests other diets were used that will be indicated later. The condition of the animals was observed regularly, body weight was noted, and a simple saline smear of material scraped from

the end of the colon was examined microscopically. Various species of coccidia and trichomonads were present in the intestine but were not found to be the cause of significant pathology in these animals. No natural amebic infections were found in the rabbits.

A laparotomy was performed on the animals under nembutal anesthesia and they were inoculated intracecally with trophozoites of *E. histolytica* strain 200, which was originally isolated by Tobie in 1947 from a human case of acute amebic dysentery. The history of strain 200 was described by the same worker, (Tobie, 1949). During the first year, the maintenance in Locke's-egg-rice starch (LER) medium was interrupted by a passage through a dog and through a rabbit, then it was continued in culture for almost another year. The series of cultures with which the work reported herein was conducted, series 200 NRS and 200 R, were obtained subsequently as follows: 200 NRS cultures were produced by micro-isolating trophozoites from culture material and establishing them with the flora of the NRS strain. 200 R cultures were obtained by scraping an ulcer of a rabbit previously infected with strain 200 from Tobie's cultures. A harvest of several 48- to 72-hour cultures was adjusted to give counts of 5,000 to 600,000 trophozoites in the inoculum for each animal. Usually 100,000 to 300,000 amebae were given in a one ml. suspension. Some rabbits received inocula consisting of suspensions of the bacterial associates or culture media alone without subsequently developing any microscopic lesions. The animals were obtained in groups of 12 or more, all of which were inoculated with approximately similar numbers of amebae from the same harvest. Usually, treatment of one-half of the rabbits started 1 to 3 days before inoculation, on the day of inoculation, or 2 to 10 days afterwards. Different doses of the drugs were given according to weight of animal once or twice a day by the oral or subcutaneous route. The treatment was continued for 1, 2, or 3 weeks. The animals were autopsied after death or were sacrificed when they became moribund; a few were sacrificed before reaching the agonal stage. Rabbits surviving the first month post inoculation were observed for a second month in order to determine survival period, delayed onset of the disease, or the occurrence of relapse. At autopsy, the number and extent of the lesions in the large intestine were described, and the presence of amebae in the lesions or in fecal material was noted. A system of grading the infections was set up on the basis of the number and kind of lesions found as follows: 0, no lesions or amebae; 1+, from 1 to 25 small lesions 0.5 mm. to 2.0 mm. in diameter and/or 1 ulcer 3 to 5 mm. in diameter, trophozoites occasionally found in lesions; 2+, from 26 to 50 small lesions and/or 2 to 10 ulcers each more than 5 mm. in diameter, amebae commonly found in lesions; 3+, ulcerated condition throughout cecum with trophozoites common in lesions but rare in anal smear; and grade 4+, ulcerated condition throughout all of large intestine, motile amebae common in the wall of the large intestine and in the anal smears.

The average degree of infection (A.D.I.) was calculated on the basis of the grades of infection of all the animals within the group. In addition, the average survival period (A.S.P.) of the animals which developed the infection was calculated for each group. The absence of lesions containing *E. histolytica* insofar

as could be determined by smears and stained sections was considered the essential criterion of the curative value of any drug.

DESCRIPTION OF INFECTION

The infection in the rabbits resulting from the intracecal injection of the *E. histolytica* culture 200 R and 200 NRS developed into an acute ulcerative amebiasis similar to that described by Tobie (1949). Of 126 animals inoculated with culture 200 R, 84.1 per cent developed the infection, while 92 per cent of 91 animals inoculated with culture 200 NRS became infected. The average degree of infection of these two groups was 3.3 and 3.2 respectively out of a possible 4; in other words they were heavy infections for the most part. The rabbits with heavy infections developed a diarrhea from the fourth to the tenth day which usually continued until the death of the animal. During the diarrhea, the stools of the animal gradually changed from a liquid feces to mucus with cellular debris but little macroscopic blood. In the latter stage, active amebae were passed by many of the animals. It was found that the prepatent period ranged from 6 to 12 days with an average of 8 days, while the patent period continued from 1 to 8 days with an average of 4 days and terminated with the death of the rabbit. About the time of the onset of the diarrhea, the animals went "off feed," they became weak, poor in appearance, and finally moribund. The body weight of many of the animals was reduced by one-third to one-half from time of inoculation to death.

Clean animals were housed with sick animals and allowed to ingest soiled food for a long period of time; they did not develop the disease, nor did those which had received capsules containing liquid feces and mucus from severely infected animals.

Autopsy findings indicated that the diarrhea began at the time that the lesions first became evident in the large intestine. Upon examination of the animals from the fourth to the eighth day after inoculation, ulcers up to 0.5 cm. in diameter or groups of small punctate lesions about 1 mm. in diameter were usually found in the area of the ileo-cecal valve or at the base of the appendix. After the eighth day, ulcers with diameters of more than one centimeter were seen at the points mentioned and elsewhere in the cecum. The lesions started as small foci which increased in number, then enlarged and finally coalesced, involving most of the large intestine.

This was the type of disease development seen in most (about 80 per cent) of the cases which became infected after inoculation with the rabbit-passed strain 200 R. All the animals died from the acute infection. For the 106 animals infected with 200 R, the survival time post inoculation varied from 7 to 61 days while the average survival period (A.S.P.) was 16.0 days. The survival time of 78 rabbits infected with 200 NRS ranged from 6 to 36 days while the A.S.P. was 14.6 days.

Since approximately 15 per cent of animals maintained on the diet of oats, corn, and bran remained refractory to infection with strain 200 R, two other diets were offered to a number of animals. These diets were Purina laboratory

chow which included not less than 23 per cent crude protein and a laboratory diet consisting of 2.5 per cent fat, 50 per cent dry skimmed milk, 25.7 per cent sucrose, 17.8 per cent alfalfa leaf meal, and 4 per cent salt. The latter diet was about 22 per cent protein while the grain diet was only about 11 per cent protein. No greater rates of infection were obtained in rabbits fed these modifications of the diet.

With regard to the age of the animal and susceptibility to infection, in 4 tests, animals 4, 6, 7, 8, and 12 weeks old were each given about 300,000 amebae from the same harvest. The percentage of infections and the A.D.I. in the older animals were the same as in the younger animals. Two and a half to 3 months after the first inoculation, 3 rabbits which failed to develop the disease, as confirmed by exploratory laparotomy, were inoculated a second time. Within 13 to 17 days, 2 of the 3 animals developed acute amebiasis and died soon thereafter. This indicates that some condition had existed temporarily which prevented the infection from establishing itself at the time of the first inoculation.

TREATMENT RESULTS

The chemotherapeutic value of various drugs in this experimental acute ulcerative amebiasis was tested by treatment of rabbits inoculated as described above. Table 1 summarizes the results of oral administration of the commonly used organic iodine compounds. The data on the number of animals found to be free of the infection at autopsy as compared to the number infected in both treated and control groups are given, as well as the average survival period (A.S.P.), and the average degree of infection (A.D.I.).

When initiated before inoculation, a dose of Diodoquin near the equivalent of the human daily dose (20 mg./kg.) did not prevent the development of fatal amebiasis in 20 animals. Even with 100 mg./kg., started on the day of inoculation, all of 8 rabbits became infected and showed no significant decrease in the A.D.I. However, 200 to 300 mg. Diodoquin/kg. beginning on the day of inoculation prevented 8 of 12 rabbits from contracting amebiasis and decreased the A.D.I. but did not appreciably lengthen the survival period, probably because of drug toxicity. No more animals were found free of infection when treated with Vioform, chiniofon, and Diodoquin starting 2 to 10 days after inoculation than were found among the controls. Furthermore, the survival period and grade of infection were not improved in the treated group.

As can be seen in Table 2, 20 mg. carbarsone/kg. started 2 days after inoculation and given orally each day for 7 days prevented the infection in 2 of 4 rabbits which survived longer than the controls and showed lighter infections at autopsy. In another series in which the disease was allowed to develop for 8 to 10 days before oral carbarsone was administered, the animals died as rapidly as the controls and with infections almost as heavy. However, one of the animals of this series, found by laparotomy to be heavily infected before treatment, became free of the infection.

A series of daily intramuscular injections of emetine hydrochloride started 2 days before inoculation resulted in 6 animals free of amebiasis of the 15 treated.

This group survived a longer period and showed a lower degree of infection than the controls. When emetine therapy was begun on the day of inoculation only 2 of 10 rabbits were found infected, and these to a light degree. However, the treated animals died of drug toxicity within the first week, before microscopic amebic ulceration developed, which would explain the low percentage and degree of infection. The administration of emetine from 3 to 10 days after inoculation did not alter the course of the disease to any appreciable extent.

TABLE 1

Results of treatment with iodinated quinolines of rabbits inoculated with Endamoeba histolytica

DRUG	TREATMENT			RESULTS		
	Rx start (day before or after inoc.)	Total daily dose	Days	No. clear*/ No. inoc. animals	A.S.P. (days post inoc.)	A.D.I.
		(*mg./kg.*)				
Diodoquin	−3	22	5	0/5	13.0	3.2
Control	—	—	—	0/5	10.7	3.0
Diodoquin	−2	30	20	0/15	20.0	4.0
Control	—	—	—	1/5	14.7	4.0
Diodoquin	0	100	7	0/8	18.0	3.1
Control	—	—	—	0/8	15.5	3.5
Diodoquin	0	200	7 & 15	4/6	11.0	1.0
Control	—	—	—	0/4	12.5	4.0
Diodoquin	0	300	7	4/6	12.5	1.1
Control	—	—	—	1/6	14.0	3.0
Diodoquin	2 to 10	80–200	14	1/14	14.4	3.0
Chiniofon	2 to 10	80–200	14	2/12	14.9	2.6
Vioform	2 to 10	80–200	14	0/14	15.2	3.3
Control	—	—	—	3/19	17.5	3.4

* The term "clear" is used to describe animals whose intestines were free of ameba-containing ulcers at autopsy.

Table 3 summarizes the results of the oral administration of the two antibiotics, aureomycin, and terramycin. Six animals which began receiving 200 to 400 mg. aureomycin hydrochloride/kg. from the seventh to the tenth day were not cured. The A.D.I. of these animals was higher than that of the untreated controls. However, when 50 and 100 mg. aureomycin/kg. were given daily starting with the day of inoculation or on the second to the fourth day after inoculation, amebiasis did not develop in 18 of 21 rabbits. With one exception, the untreated controls all became infected and died within 2 weeks post inoculation. Of the 14 animals which were started on the aureomycin treatment on the day of inoculation, 3 developed a low grade infection. The survival period of the rabbits placed on aureomycin therapy within 4 days after inoculation was almost twice that of the untreated controls.

A similar response was observed in the animals given terramycin hydrochloride soon after inoculation. Sixteen of 27 rabbits placed on terramycin therapy within

5 days after inoculation were found free of the infection. In contrast, cure of amebiasis was not obtained in animals started on the therapy as late as the seventh day, when the disease had already been established.

TABLE 2

Results of treatment with carbarsone and emetine of rabbits inoculated with Endamoeba histolytica

DRUG	TREATMENT			RESULTS		
	Rx start (day before or after inoc.)	Total daily dose	Days	No. Clear/ No. inoc. animals	A.S.P. (days post inoc.)[1]	A.D.I.
		(*mg./kg.*)				
Carbarsone	2	20	7	2/4	18.5+	1.7
Control	—	—	—	0/4	15.0	4.0
Carbarsone	8 to 10	20 to 66	10	1/8	13.0	1.9
Control	—	—	—	1/5	16.4	2.8
Emetine	−2	2.1	12	6/15	18.3	2.0
Control	—	—	—	1/5	13.5	3.2
Emetine	0	2.5 & 5.0	10	8/10	7.0	0.2
Control	—	—	—	0/7	8.5	3.7
Emetine	3 to 10	2.1	10	5/13	14.5	1.5
Control	—	—	—	2/7	12.8	2.8

[1] + indicates some animals were sacrificed so that an exact A.S.P. is not calculable.

TABLE 3

Results of treatment with antibiotics of rabbits inoculated with Endamoeba histolytica

DRUGS	TREATMENT			RESULTS		
	Rx start (days before or after inoc.)	Total daily dose	Days	No. Clear/ No. inoc. animals	(Days post inoc.) A.S.P.[1]	A.D.I.
		(*mg./kg.*)				
Aureomycin	0	50 & 100	10 & 14	11/14	31.0	0.3
Control	—	—	—	1/17	15.0	3.9
Aureomycin	2 to 4	50 & 100	10 & 18	7/7	20.7	0.0
Control	—	—	—	0/8	11.6	3.9
Aureomycin	7 to 10	200 to 400	10	0/6	18.1	3.7
Control	—	—	—	1/6	28.0	1.8
Terramycin	0	25 & 50	7 & 14	11/20	29.0	0.7
Control	—	—	—	2/15	14.1	3.4
Terramycin	2 to 5	12.5	10 & 14	5/7	29.5+	0.4
Control	—	—	—	1/9	17.5+	3.3
Terramycin	7	25	14	1/6	20.8	3.0
Control	—	—	—	1/6	21.5	3.1

[1] + indicates some animals were sacrificed so that an exact A.S.P. is not calculable.

Although these two antibiotics were able to prevent the development of amebiasis in rabbits, the administration of the doses indicated was accompanied by signs of toxicity. After one week of treatment, most of the infected or non-

infected animals developed an enlarged abdomen, which in most cases existed until the death of the animal. At autopsy, it was found that the cecum filled most of the peritoneal cavity. The contents of the cecum were fluid in nature. Uninfected young rabbits on the deficient grain diet, given 12.5 mg. of terramycin or aureomycin/kg. body weight daily for 10 days, developed the same abdominal distension. Four animals given this dose of terramycin died from 39 to 51 days after start of treatment whereas 2 of 5 rabbits which received the same dose of Aureomycin died on the forty-third day, and untreated animals on the same deficient diet survived an average of 94 days.

DISCUSSION

Since it was found that the acute ulcerative amebiasis in rabbits responded to early treatment with some of the known amebicides, it is of interest to compare the results reported here with those obtained in chemotherapy of a similar type of disease in humans. It is thought that the treatment of acute ulcerative amebiasis in man reported by Armstrong *et al.* (1949, 1950) is most comparable to the present series. These investigators found that the administration of 30 mg. Diodoquin/kg. daily for 20 days resulted in cure of 29 of 50 persons, whereas practically this same dose did not cure rabbits even when the drug was started before inoculation. However, when doses 7 to 10 times larger were received by the rabbits, they did not develop fatal infections. Since Armstrong *et al.* cured only 58 per cent of their cases with the described regimen, it is possible that if a somewhat larger daily dose had been given, the treatments might have resulted in greater success. Emetine at the rate of almost 2 mg./kg. daily for 10 days resulted in 50 per cent success in 50 human cases, whereas in the present tests this same dosage started before inoculation prevented 20 per cent of the rabbits from contracting the disease. Larger doses of emetine killed the animals within the first week of treatment.

When 20 mg. carbarsone/kg. was administered to rabbits starting 2 days after inoculation, one-half of them were cleared. This is practically the same percentage of cure obtained in treatment of humans with 10 or 15 mg. carbarsone per kilogram body weight for 20 days.

Recent reports have indicated that some of the antibiotics are more active amebicides than previously used drugs. McVay *et al.* (1949, 1950) were first to demonstrate activity of aureomycin in amebic infections in humans and cure was claimed by these workers. In the series of Armstrong *et al.*, 94 per cent of 50 persons with acute amebic dysentery were cured by a dose of 15 to 20 mg. aureomycin/kg. for 15 days, but a relapse one month later occurred in 25 per cent of the cases. Fifty mg. of this antibiotic/kg. per day prevented the infection in rabbits which received treatment starting as late as the fourth day after inoculation. Similarly, rabbits responded to early treatment with terramycin; approximately 50 per cent of such treated animals did not develop the disease.

Ruiz Sanchez *et al.* (1950) reported the satisfactory treatment of 8 clinical cases of amebic dysentery by use of 30 to 50 mg. terramycin/kg./day for 4 to 6 days, and there was no relapse observed during a period of 14 to 30 days. In

addition, Most *et al.* (1950) and Tobie *et al.* (in press) have reported the value of terramycin in treatment of asymptomatic and mildly symptomatic cases of colonic amebiasis. However, during the aureomycin and the terramycin treatment of rabbits on the deficient diet, many of the animals showed signs of toxicity in a marked distension of the cecum which continued for several days and resulted in perforations of lesions in many of the infected rabbits. Marked abdominal distension has not been reported for man under treatment with either of these antibiotics.

It is thought that there is sufficient similarity in response to chemotherapy between acute ulcerative amebiasis in the rabbit and in man to conclude that the rabbit can be used as a test animal for screening drugs. However, in order to test for drug activity against so fulminating an amebiasis, it is necessary that treatment be initiated within 4 days after inoculation of the rabbit with trophozoites of *E. histolytica*.

CONCLUSIONS

1. An acute ulcerative amebiasis was produced in 87.5 per cent of 217 young rabbits by intracecal inoculation with trophozoites of either of two cultures of *E. histolytica* originally isolated from a case of acute human amebiasis.

2. Diarrhea and loss of weight were first observed from 4 to 10 days after inoculation, while trophozoites were first found in anal smears on the sixth to the twelfth day, and they were passed for 1 to 8 days. No cysts were found, and the liquid stools were not infective to clean animals.

3. All the animals died of the infection which usually terminated with a generalized ulceration of the cecum. The average survival period was 15 days.

4. Twelve-week-old rabbits developed the infection as readily and to the same degree as did four-week-old rabbits.

5. Animals refractory to the first inoculation became infected upon a second challenge.

6. Groups of rabbits inoculated with *E. histolytica* were prevented, all or in part, from developing a fatal infection by early and intensive treatment with carbarsone, emetine, aureomycin, terramycin, or large amounts of Diodoquin.

7. The rabbit is a satisfactory test animal for the demonstration of antiamebic activity of chemicals against acute ulcerative amebiasis if treatment is undertaken within 4 days after intracecal inoculation with trophozoites of *E. histolytica*.

REFERENCES

In future issues of this Journal, references will be cited by date and not by number.

ANDERSON, H. A., HANSEN, E. L., SAH, P. T. P., AND CAFISO, J. R.: Amebacidal and pharmacologic activities of carbarsone oxide (p-carbamidophenylarsenous oxide) and its dithiocarboxymethyl and dithiocarboxyphenyl derivatives. *J. Pharm. Exp. Therap.* **91:** 112–123, 1947.

ANDERSON, H. A. AND ANDERSON, J. V. D.: Antibiotics against amebiasis in Macaques. *Am. J. Trop. M.* **30:** 193–215, 1950.

ARMSTRONG, T. G., WILMOT, A. J., AND ELSDON-DEW, R.: The treatment of amoebic dysentery in the Bantu African. *Trans. Roy. Soc. Trop. Med. & Hyg.*, **42:** 597–604, 1949.

ARMSTRONG, T. G., WILMOT, A. J., AND ELSDON-DEW, R.: Aureomycin and amoebic dysentery. *Lancet* **259:** 10–12, 1950.

CARRERA, G. M. AND FAUST, E. C.: Susceptibility of the guinea pig to *Endamoeba histolytica* of human origin. *Am. J. Trop. M.*, **29:** 647–667, 1949.

CLAMPIT, J. M.: Comparative therapy of *Endamoeba histolytica* infections in the kitten. *Am. J. Trop. M.* **28:** 289–294, 1948.

DENNIS, E. W., BERBERIAN, D. A., AND HANSEN, S. S.: Amoebicidal activity of bismuthoxy p-N-glycolylarsanilate and 7-iodo-4-(1-methyl-4-diethylaminobutylamino) quinoline diphosphate. *Am. J. Trop. M.* **29:** 683–689, 1949.

HASKINS, W. T., LUTTERMOSER, G. W., AND BEADY, F. J.: The physiological disposition of diodoquin, vioform, and chiniofon in the rabbit as determined with radioiodine. *Am. J. Trop. M.*, **30:** 599–612, 1950.

HASKINS, W. T., AND LUTTERMOSER, G. W.: Spectrophotometric determination of 8-quinolinol. *Anal. Chem.* **23:** 456, 1951.

JONES, W. R.: The experimental infection of rats with *Endamoeba histolytica;* with a method for evaluating the anti-amebic properties of new compounds. *Ann. Trop. M. &. Parasit.* **40:** 130–140, 1946.

JONES, W. R.: The therapeutic action of some known amoebicides in rats. Brit. J. Pharmacol. **2:** 217–220, 1947.

JONES, W. R.: The experimental chemotherapy of amoebiasis. *Proc. Fourth Inter. Cong. Trop. M.* **2:** 1088–1096, 1948.

MCVAY, L. R., LAIRD, R. L., AND SPRUNT, D. H.: A preliminary report of the successful treatment of amebiasis with aureomycin, *Science*, **109:** 590–591, 1949.

MCVAY, L. R., LAIRD, R. L., AND SPRUNT, D. H.: The treatment of amoebiasis with aureomycin. *S. M. Jour.* **43:** 308–313, 1950.

MOST, H. AND VAN ASSENDELFT, F.: Laboratory and clinical observations on the effect of terramycin in the treatment of amebiasis. *Ann. N. Y. Acad. Sc.* **53:** 427–428, 1950.

RUIZ SANCHEZ, F., RIEBELING, R., AND ARREOLA, C.: El tratamiento de la disenteria amebiana con terramycin; nota preliminar. *Medicina Mex.* **30:** 365–369, 1950.

TAYLOR, D. J., GREENBERG, J., AND COATNEY, G. R.: Experimental infection of guinea pigs with *Endamoeba histolytica. J. Parasit.* **35:** (Suppl.) 24, 1949.

TAYLOR, D. J., GREENBERG, J., HIGHMAN, B., AND COATNEY, G. R.: Experimental infection of guinea pigs with *Endamoeba histolytica. Am. J. Trop. M.* **30:** 817–828, 1950.

THOMPSON, P. E. AND LILLIGREN, B. L.: Chemotherapy of experimental *Endamoeba histolytica* infection in dogs. *Am. J. Trop. M.* **29:** 323–336, 1949.

THOMPSON, P. E., DUNN, M. C., BAYLES, A., AND REINERTSON, J. W.: Action of chloramphenicol (chloromycetin) and other drugs against *Endamoeba histolytica in vitro* and in experimental animals. *Am. J. Trop. M.* **30:** 203–215, 1950.

TOBIE, J. E., MOST, H., REARDON, L. V., AND BOZICEVICH, J.: Laboratory results in the efficacy of terramycin, aureomycin, and bacitracin in the treatment of asymptomatic amebiasis. *Am. J. Trop. M.* **31:** 414–419, 1951.

TOBIE, J. E.: Experimental infection of the rabbit with *Endamoeba histolytica. Am. J. Trop. M.* **29:** 859–870, 1949.

THE DYSENTERIES IN THE ARMED FORCES

ALBERT V. HARDY,[1] RICHARD P. MASON,[2] AND GERALD A. MARTIN[3]

"Joint Dysentery Unit", 64th Field Hospital, Far East Command

History abounds with examples of dysentery as a disease of wars. "No major military campaign has been free of its ravages" (Felsen). But it is one with which our medical and sanitation officers cannot become familiar in their civilian experiences. The filth-borne diseases are fast disappearing in this country and our young clinicians and public health workers, who predominate in the medical services of the Armed Forces, scarcely encounter cases of typhoid fever or dysentery during their training. There is even less opportunity for contact with epidemics of enteric infections. Certain aspects of an epidemic in Korea involving prisoners of war, will be considered here as illustrative of the peculiar nature of the problem of dysentery in the Armed Forces.

The most unusual feature of the epidemic was its size. An outbreak of enteric infections with 161 hospitalized cases with a fatality rate of 9 per cent, and some 800 milder nonhospitalized cases, would be regarded as a major epidemic. In the Korean outbreak an average of this number of cases and deaths occurred each day throughout a four month period. Moreover, the outbreak continued at a lower level for about one year. There were, in other words, some 150 major epidemics compressed into one.

The responsibility of the United Nations for the North Korean prisoners of war was a small part of its broad activities. Diarrheal disease was present everywhere. The civilian refugees, for whose health and welfare the Armed Forces of the United Nations had official concern, lived under poor sanitary conditions; the prisoners of war had excellent facilities by comparison. The amount of diarrheal diseases occurring endemically among them, if the cases and deaths had been reported, probably would have far exceeded that among the prisoners. Further, South Korean officers from a near-by battalion sought the assistance of our unit in the diagnosis and treatment of diarrheal disorders which were highly prevalent among their troops. Even among the American personnel, particularly in the Navy, some units were temporarily ineffective due to a general and high prevalence of diarrheal disease. During and since World War II, the Armed Forces of the United States have lived and fought in countries in which the diarrheal diseases are highly prevalent—more prevalent probably than they have been in this country at any time during our entire history. Today in military medicine the infectious diarrheal diseases continue to be highly important.

[1] Member, Commission on Enteric Infections, Armed Forces Epidemiological Board and Director, Bureau of Laboratories, Florida State Board of Health.

[2] Colonel, M. C., and Director, 406th Medical General Laboratory, Tokyo.

[3] Lieutenant, M. C., U. S. N., Fleet Epidemic Disease Control Unit, No. 1. Dr. Martin died in a plane accident in Japan in September, 1951.

Diagnosis is the first problem in any epidemic of enteric infections. In this country this is relatively simple, as for example, in institutional outbreaks. A representative sample of the cases, when they are examined, ordinarily are found positive for one pathogen only. The unexamined cases may be assumed to be due to the same cause. Such will be the findings in an epidemic occurring in a population among whom the infections normally are rare. The situation in Korea was markedly different. Each day among cases admitted to hospital, there would be an admixture of cases of amebic dysentery, bacillary dysentery due to several types of *Shigella*, some salmonellosis and the inevitable cases of diarrhea of undetermined etiology. Thus, in a population with a high endemic incidence of dysentery, it becomes increasingly important to do adequate diagnostic studies on each individual case.

Laboratory studies are essential for the reliable diagnosis of acute diarrheal diseases and under the above conditions large numbers of diagnostic tests are required daily. Hence, laboratories serving the Armed Forces in the field need to be prepared for volume work in enteric bacteriology and parasitology. Most bacteriologists are trained where few fecal cultures are performed and each suspicious organism is studied with an array of biochemical and serological tests. Similarly, in parasitology, students have been impressed with the need for repeated and time-consuming tests. Only a few cases per day can be examined by such methods. Actually this approach does not meet the need in an Armed Forces installation.

Three laboratories had been concerned with the study of the dysentery occurring among the prisoners of war by the methods ordinarily employed. Specimens were collected on the wards and sent to the laboratory. The number per day was limited to 25 as a maximum. The importance of fresh specimens had been emphasized, though it was learned later that the specimens were collected ordinarily during the night and submitted in the forenoon. These methods had revealed an occasional case of amebic dysentery and some 25 bacteriologically positive cases per week.

When we first had contact with the problem, the case load had dropped markedly. Two simple changes were instituted at that time. The parasitology technicians with their microscopes were moved to the hospital. There was no laboratory, no electricity, no table—just large boxes for the microscopes and smaller ones to sit on, but fecal specimens were brought to the microscopists immediately on passage. Amebic dysentery changed from a rare sporadic infection to an epidemic disease with more than fifty severe acute cases per week. In bacteriology the use of the rectal swab was begun. On the second day 89 patients were cultured and 64 were found positive for an enteric pathogen. It soon became routine to take 300 to 400 cultures daily, which included the follow-up on treated cases. It was not unusual to report more than 50 positives per day. By these changes, without any increase in the technical staff—but with additional glassware and media—the number of cases diagnosed by dependable laboratory findings was multiplied many times. The objective of a service laboratory in the Armed Forces must be to provide reliable diagnostic aid in as many cases as practicable. To attain this goal for enteric infections, the

essential requirements include fresh specimens, preferably direct from the patient, a careful selection of media and biochemical tests, an adequate supply of dependable diagnostic antisera and senior laboratory workers with experience in organizing and handling volume work.

The clinician as well as the bacteriologist must be able also to adjust his procedures to provide needed services for large numbers of patients. Sigmoidoscopic examinations, for example, are of substantial assistance in the diagnosis of dysentery. In our experience in Korea at first two or three sigmoidoscopic examinations were a major accomplishment. It was found practicable next to do initial examinations on all cases positive for *E. histolytica* trophozoites, likewise follow-up observations to determine the response to therapy. A little later with additional instruments and better facilities for their sterilization, the severe cases which were negative bacteriologically were referred for diagnostic sigmoidoscopic studies. This led to the final step of adopting as a routine the sigmoidoscopic examination of all patients admitted to the service. The daily admissions were then between 50 and 90 and follow-up examinations averaged some 35 daily. It became a not too strenuous routine for two physicians and the prisoners of war assistants to make 100 sigmoidoscopic observations daily. The record reached 142 examinations in one day.

There is an understandable reluctance to replace the exhaustive and limited studies of pure science with the volume work required in applied science. The former seems exact and satisfying, the latter appears less so. Actually, well organized volume work need not compromise reliability in the single case, and the resulting findings provide a much more reliable picture of conditions in the group as a whole. If communicable diseases occurring epidemically—or endemically—are to be handled effectively, a change of viewpoint is required. The group more than the single case must be the object of study. To attain this goal some modification of training procedures appears indicated. Laboratory workers in particular require the opportunity to learn the method of handling volume work. There are obvious difficulties in providing this training experience, but one possibility is suggested. The public health laboratories, of necessity, must develop efficient procedures for handling large numbers of specimens. The potential value of these laboratories for training purposes has not been appreciated.

A second striking feature of the epidemic under study was the apparent atypical nature of findings. The dysenteries seen in Korea differed widely from the shigellosis and amebiasis encountered in this country. It has been emphasized repeatedly that the usual manifestation of *Shigella* infections in this country is a simple diarrhea. The infection in young adults as observed here is a relatively mild and transient disorder. Gross bloody exudate is unusual. In Korea by contrast, the disease was the classical bacillary dysentery. In case after case the stools were nothing but a mass of bloody mucopurulent exudate. It has been recognized in other infections that the nature of a disease may vary from area to area and time to time. The observations made in this epidemic emphasize that this can be true for bacillary dysentery.

The clinical features of the acute amebic infections were not atypical but the

laboratory findings were unusual. The characteristic motile *E. histolytica* was present in the exudate of some of these cases in amazingly large numbers. Microscopically, there would appear to be nothing but a squirming mass of motile amebas. Accurate counts were impossible, but it was repeatedly concluded by multiple observers that there would be more than 100 *E. histolytica* per high power field in these exceptional preparations.

The bacteriological studies of the bacillary infections provided a challenging and exasperating array of problems. The identification of shigellosis in adults in this country is a comparatively simple laboratory procedure. The organisms are ordinarily present in substantial numbers, the colorless colonies are uniform in appearance and contrast strikingly with the red colonies of the lactose-fermenting organisms. The cultures in Korea however, had the widest variety of suspicious colonies. By inspection of plates one could not select from this assortment those which probably would prove to be *Shigella*. Moreover, in positive cases the pathogens frequently were present in small numbers only. Of multiple simultaneous cultures on the same case some would be found positive and others not. Two experienced workers picking the same lot of plates would identify about the same number of positives, but each would miss some which were found by the other. There were further problems in salmonellosis. Paratyphoid A was widely prevalent. Elsewhere enrichment media are accepted as an essential in the isolation of *Salmonella* but in Korea neither selenite or tetrathionate had any value as an enrichment for *Salmonella paratyphi A*.

It is clearly evident that enteric infections as encountered in the Armed Forces abroad may differ widely from those seen at home.

The Korean outbreak demonstrated again that an epidemic situation provides an opportunity to accumulate valuable scientific data very rapidly. The evaluation of the relative efficacy of antibiotics in amebiasis and bacillary dysentery was the major interest at this time. Within a six month period some 650 cases of acute amebic dysentery were observed on 24 different therapeutic or dosage schedules with close clinical and laboratory follow-up during therapy and for four weeks thereafter. The uniformity of conditions and type of case provided unusually favorable conditions for comparative studies. Moreover, some 1600 cases of proven or suspected bacillary dysentery were followed on 18 different treatment or dosage schedules. The studies were a sound type of clinical inquiry which were in the best interest of the patients themselves.

The need for assistance in unusual outbreaks of communicable disease has been recognized and appropriate plans developed. When this epidemic was reported, the information was made available to the Chairman of the Armed Forces Epidemiological Board and through him to the Commission on Enteric Infections (The Board and its Commissions are civilian consultants who work in association with designated representatives from the medical Services of the Armed Forces). A plan of action was formulated, recommended and promptly approved. A civilian member of the Commission and a senior officer from the Army Medical Service Graduate School proceeded to the site of the epidemic to investigate, with authorization to call for additional assistance and supplies

if needed. A broad cooperative activity rapidly developed. The Fleet Epidemic Disease Control Unit with its well equipped floating laboratory and trained staff was already there, and permitted the immediate initiation of indicated studies at the site of the epidemic. The medical officers in charge of preventive medicine and laboratory services in the theatre gave every assistance. That part of the clinical staff of the 64th Field Hospital which was providing medical care to dysentery cases became part of what was designated "The Joint Dysentery Unit". Scientists working in the fields of bacteriology, parasitology, epidemiology, sanitary engineering and investigative clinical medicine came to join the Unit for periods of two or more months as working consultants. The Joint Dysentery Unit took full charge of the problem, including managing the large and active dysentery service in the hospital. This Unit had value beyond the contribution of its individual members. Its requests for services or supplies were given high priority. Facilities previously unavailable were soon at the disposal of the Unit. There is therefore, an effective mechanism for bringing to a problem from civilian or Service sources both expert assistance and needed facilities, if the problem justifies special study or unusual control activities.

The assistance provided in this epidemic probably was less effective than it might have been. The epidemic had been in progress four months before its occurrence was made known to responsible officers in Washington. It is doubtful whether the program which started in May could have been applied in December or January, but prompt reporting would have involved a sharing of responsibility. Moreover, in the usual outbreak of limited duration, early reporting and immediate action are required if an epidemic is to be rapidly controlled or adequately studied.

There is a particular need for consultants in the field of infectious diseases in the Armed Forces. A high proportion of medical officers over-seas are young, energetic and resourceful but with little experience other than that derived from studies on predominantly noninfectious illnesses encountered in academic and hospital training. They have had virtually no opportunity to learn the problems of the common, prevailing and epidemic infections of tropical areas. This recent experience suggests that with advantage, needed guidance can be provided through the intimate cooperation of civilian commission members and senior medical officers of broad experience in preventive military medicine.

Epidemics of dysentery encountered in the Armed Forces may be of unprecedented size, the clinical and laboratory features may be atypical, and the opportunities for study may be unexcelled. There are simple though essential requirements for the handling of these major problems. For effective action, prompt reporting is as essential in military as in civilian practice. Consultative assistance in the clinical, laboratory and epidemiological field may be required. A mobile laboratory may be essential for the highly important early studies of a major epidemic. Our experience in Korea calls attention to the value of the commissions of the Armed Forces Epidemiological Board. A more frequent use of this available assistance in the field may be recommended.

CORRECTION

Through an unfortunate mistake, the illustrations in the article "*Endamoeba gingivalis* in pulmonary suppuration" by Sutliff, Green and Suter, in the November 1951 issue of the American Journal of Tropical Medicine on page 718, were transposed and placed over the wrong descriptions.

IMPROVED MODEL, FANZ AUTOMATIC

MICROTOME KNIFE SHARPENER

With automatic knife lifting and reversing device

An early model of this Sharpener has been in constant use for eighteen years. During this period approximately 10,000 knives have been reconditioned without repairs to the Sharpener except for the occasional replacement of a worn belt.

7203.

MICROTOME KNIFE SHARPENER, Fanz Automatic, Improved Model. With simplified, enclosed motor drive and with Miller automatic knife lifting and reversing device. Takes knives up to 300 mm in length and 12 mm thick. Provides more nearly perfect cutting edge than skillful hand honing and stropping.

A revolving glass disc, 21 inches diameter, is driven against the knife which is automatically swung to and fro in an arc through the center of the disc surface. The automatic attachment holds the knife in contact with the glass disc for approx. 27 seconds, during which period it makes 4.5 reciprocating cycles, and is then lifted and turned over in approx. 8 seconds and the process repeated on the opposite side. A scale on the automatic attachment provides for precise, reproducible setting of the angle of the bevel. Metal housing is 25 inches square × 11 inches high and is finished in gray crystal lacquer.

7203. **Microtome Knife Sharpener, Fanz Automatic, Improved Model,** as above described, with Miller knife lifting and reversing device, complete with glass disc, drip deflector, glass reservoir, 2 liter capacity, rubber and Tygon tubing connections, and 1 lb. each of white rouge and castile soap. For 115 volts, 50 or 60 cycles, a.c. **633.10**

More detailed information sent upon request.

ARTHUR H. THOMAS COMPANY

LABORATORY APPARATUS AND REAGENTS

WEST WASHINGTON SQUARE

PHILADELPHIA 5, PA.

Teletype Services: Western Union WUX and Bell System PH-72

(In writing to advertisers, please mention the journal—it helps.)

REVISED and EXPANDED SECOND EDITION
THE

Author-Publisher-Printer Complex

By ROBERT S. GILL

Modifications made necessary by changes in production methods • More complete information on many points of importance • New chapter on literary property and inheritance • Complete rearrangement of material for more logical order • Addition of index to make material more accessible 153 pp. 9 figs. $1.50

"Can be read with pleasure and profit by the most experienced and blasé writer."
—*American Journal of Obstetrics and Gynecology*

THE WILLIAMS & WILKINS CO.

Mt. Royal and Guilford Aves. Baltimore 2, Maryland

INDIAN JOURNAL OF MALARIOLOGY

Published quarterly under the authority of the Indian Research Fund Association. Illustrations in Colour and Half-Tone, Diagrams and Charts.
The only medium that publishes results of Malaria Research Work of leading Malariologists in India and the East.

Annual Subscription Rs. 12 (payable in advance). Single copy Rs. 5.

Detailed list of all back numbers available may be had on request.

All Business Communications respecting advertising in or subscriptions to the above journal should be addressed to—

The Manager
Thacker, Spink & Co. (1933), Ltd.
Post Box 54, Calcutta

JOURNAL OF PARASITOLOGY

Official Organ of American Society of Parasitologists

A medium for the publication of papers on Animal Parasites (Protozoa, Helminthes, Arthropoda).

It also publishes the program and abstracts for the annual meeting of the American Society of Parasitologists in a December Supplement.

Regular numbers appear in February, April, June, August, October and December.

Subscription price, $7.50 the year (Foreign, (7.75).

Index (Author-Subject-Host) to volumes 1–25, 1914–1939, now available, $2.00 postpaid.

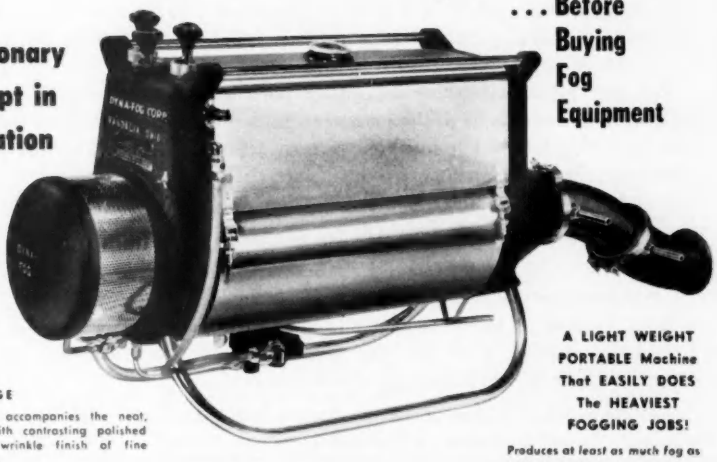
HORACE W. STUNKARD
CHAIRMAN, EDITORIAL COMMITTEE

Journal of Parasitology
New York University, University Heights, New York 53, N. Y.

(In writing to advertisers, please mention the journal—it helps.)

investigate the new Dyna-Fog JET FOG GENERATOR ... Before Buying Fog Equipment

A Revolutionary New Concept in Fog Generation



100% JET OPERATED

APPEARANCE BUILDS PRESTIGE

Professional prestige accompanies the neat, functional design, with contrasting polished chrome and black wrinkle finish of fine scientific instruments.

A LIGHT WEIGHT PORTABLE Machine That EASILY DOES The HEAVIEST FOGGING JOBS!

Produces at least as much fog as 650 lb. and heavier machines.

The MOST ECONOMICAL and PRACTICAL Applicator for ALL TYPES of INSECTICIDAL FOGGING

COMMUNITIES	FAIRS	AUTO COURTS	HATCHERIES
MUNICIPALITIES	RESORTS	RACE TRACKS	GREENHOUSES
TOWNSHIPS	ESTATES	HOTELS	FACTORIES
PARKS	HOMES	COUNTRY CLUBS	WAREHOUSES
PICNIC GROUNDS	FARMS	BEACHES	SHIPS' HOLDS
DRIVE-IN THEATERS	RANCHES	DAIRIES	RAILROAD CARS

A BARGAIN AT $1185.00

F.O.B. Vandalia, because easily does every job that old fashioned heavy machines can do, PLUS doing work impossible except with a portable machine.

OPENS NEW FIELDS FOR FOGGING!

LIGHT WEIGHT AND PORTABLE!

Weighs only 100 pounds. Easily moved to most advantageous position for indoor or difficult location jobs. Readily removable from motor vehicles to avoid "tying up" trucks or jeeps during "off" season or between jobs.

MINIMUM MAINTENANCE COST . . MAXIMUM RELIABILITY!

Operates with only three moving parts, virtually nothing to go wrong. Requires no lubrication. Has no rotating parts. Years of dependable operation assured.

MORE AND BETTER FOG!

Laboratory tests show extremely dense fog output at rates up to 40 gallons per hour. Analysis of fog shows uniform particle size, assured by exclusive pulsating jet system of operation.

LESS HEAT APPLIED TO INSECTICIDE!

Killing power proven by laboratory test. Jet gases pulsating at sonic velocity pulverize insecticide formulaton with minimum application of heat. Less "breakdown" of insecticide. More kill per formulate dollar.

TESTED AND PROVEN IN BOTH LABORATORY AND PRACTICAL FIELD SERVICE!

In addition to laboratory tests the DYNA-FOG Jet Fog Generator has been utilized with great success in commercial pest control operations throughout the 1949 pest season.

SELECTIVE FOG PARTICLE SIZE!

Fog particle size varied by simple control offering range from 0.5 micron for dryest indoor fogs to 75 microns for wetting down dumps, swamps, etc.

Dyna-Fog Corp.
DAYTON MUNICIPAL AIRPORT
VANDALIA, OHIO

Choice distributor territories still open. Inquiries from qualified firms are invited.

THE AMERICAN JOURNAL OF

Tropical Medicine and Hygiene

THE AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE is issued bi-monthly, appearing in January, March, May, July, September, and November. One volume a year is issued.

Accepted articles for which immediate publication would seem important will be inserted in the next issue of the journal to go to press if the author is willing to pay the manufacturing cost. The insertion of such articles will not affect the publication of other manuscripts awaiting their turn, since the inserted articles will constitute additional pages to the volume without additional cost to the subscriber.

Manuscripts and Books for Review should be sent to Dr. L. W. Hackett, School of Public Health, University of California, Berkeley 4, California.

Correspondence concerning business matters should be addressed to The Williams & Wilkins Company, Mount Royal and Guilford Avenues, Baltimore-2, U. S. A.

Subscription price: $10.00 per volume, United States, and countries within the postal union; $10.50 countries outside the postal union.

New subscriptions and renewals are entered to begin with the first issue of the current volume. Should any issue of the current volume be out of print at the time the subscription order is received, the pro-rata value of such numbers will be refunded to the subscriber.

Subscriptions should be renewed promptly—To avoid a break in your series, subscriptions should be renewed promptly. The publishers cannot guarantee to supply back issues on belated renewals.

Claims for copies lost in the mails must be received within 30 days (domestic). Changes of address must be received within two weeks of the date of issue.

THE WILLIAMS & WILKINS COMPANY
BALTIMORE-2, U.S.A.

(In writing to advertisers, please mention the journal—it helps.)

AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

APPLICATION FOR MEMBERSHIP

To the Society:

The following individual interested in tropical medicine and hygiene is proposed for active membership.

(TYPE OR PRINT)

Name .....
LAST FIRST MIDDLE

Mailing Address: City ..... State ..... County .....

Date of Birth ..... Place .....

Special Field of Interest .....

Education: Institution (Graduate and Postgraduate) Degree Date

.....

.....

.....

Attach to the application a list of professional positions held, membership in scientific societies, and a list of major publications.

*Proposed by:

(1) ....

(2) ..... Signature of Applicant

*Sponsorship by two active members is obligatory.

Return this application to Dr. Quentin M. Geiman, Secretary-Treasurer, Harvard School of Public Health, 25 Shattuck Street, Boston 15, Mass., U. S. A.

(Check for $8.00 (U. S. banks requested) must accompany this application.)

for the control of insects by SPRAYING

Proper atomization plus uniform distribution are the spray characteristics that make precision-built TEEJET nozzles ideal for the spraying of insecticides. Full range of interchangeable orifice tips provide capacities for every type of chemical and every method of application. Complete nozzle includes built-in strainer with monel metal screen.

SPRAYING SYSTEMS CO.

3234 Randolph Street • Bellwood, Illinois

(Suburb of Chicago)

• INTER-CHANGEABLE ORIFICE TIPS

TRIGGER TEEJET

Quality built for hand spraying. Valve body drop forged brass with stainless steel valve stem. Self-seal packing. Valve seat replaceable. Trigger lock.

Any TeeJet tip fits the Trigger TeeJet . . . also curved and straight extensions.

• TEEJET NOZZLES BUILT IN MALE OR FEMALE CONN.

WRITE FOR TEEJET BULLETIN NO. 58

(In writing to advertisers, please mention the journal—it helps.)

NEW EDITION OF
AN OLD STANDBY

STEDMAN'S

17th Edition

Medical Dictionary

EDITED BY

NORMAN BURKE TAYLOR, M.D., F.R.C.S., *University of Western Ontario*
IN collaboration with **ALLEN ELLSWORTH TAYLOR, D.S.O., M.A.**

The seventeenth edition of this well known work, revised, enlarged and brought up to date, will prove extremely valuable not only to the medical profession but to all those concerned with the biological sciences. In addition to including new words, attention has been given to cutting out dead wood and revising old definitions. The derivations have been carefully examined and revised where necessary. A new feature is the inclusion of short biographical sketches of the principal figures in the history of medicine.

Semi-flexible binding, 1363 pp., illustrated, thumb index $8.50

THE WILLIAMS & WILKINS COMPANY

MT. ROYAL AND GUILFORD AVES., BALTIMORE 2, MARYLAND

(In writing to advertisers, please mention the journal—it helps.)

Be sure to include TIFA in your budget

TODD INSECTICIDAL FOG APPLICATOR

Carrying Underwriters' Listing Seal

Solve your mosquito problems quickly and efficiently with TIFA!—the compact, easily-handled applicator which discharges insecticides in a clean, effective fog. Adult mosquitoes *can't* escape it!

Save money, time and trouble with this amazingly efficient unit. TIFA meets your every specification . . . is backed by 10 years' experience in manufacturing fog machines and TIFA carries the Underwriters' Listing Seal.

COMBUSTION EQUIPMENT DIVISION
TODD SHIPYARDS CORPORATION
81-16 45th Avenue, Elmhurst, Queens, N. Y.

THE SERVALL ANGLE CENTRIFUGE
(SUPERSPEED)

Accepted and acclaimed by scientists throughout the world for its durability, safety features, smooth performance and efficiency. Our years of experience, and our pioneering of the high speed angle centrifuges guarantee their continued superiority.

Write for Bulletin K-11

Max. speed 13,500 r.p.m.
or R.C.F. 23,000 x G
Max. capacity 8 x 50cc

Ivan Sorvall, inc.

210 Fifth Avenue, New York 10, N.Y.

(In writing to advertisers, please mention the journal—it helps.)

NOTICE TO CONTRIBUTORS

The Journal welcomes papers which contribute to the purpose of the American Society of Tropical Medicine and Hygiene. This is the advancement of knowledge of tropical and subtropical medicine and hygiene through investigation, prevention, treatment and control of diseases and deficiencies. It draws upon medicine, biology, public health, nursing, engineering and allied fields of research and application.

The American Journal of Tropical Medicine and Hygiene combines the American Journal of Tropical Medicine and the Journal of the National Malaria Society and therefore has a special concern with the investigation, therapy and control of malaria in all parts of the world, as well as a broad interest in general parasitology and in arthropod borne diseases.

Papers are ordinarily published in the order of reception, which will entail a certain delay in their appearance because of the contractual limitation on the number of journal pages available in any year. Accepted papers which raise no questions of scientific priority may however secure earlier publication if the author or the institution sponsoring the paper will pay the publication cost, an estimate of which the Editor will be glad to obtain from the publishers. Such a subvention will be used to purchase extra pages, in excess of the regular page allocation for any issue of the Journal.

Authors are asked to cooperate by complying with certain standard practices in the preparation of their manuscripts in order to facilitate the editorial task:

Papers should be written in *English*.

Manuscripts should be typewritten using *double or triple spacing* on *one side* only of $8\frac{1}{2}$ x 11 inch paper, if available, and submitted in duplicate.

Tables should be typed on separate sheets and be serially numbered. Glossy prints of tabular material eliminate expensive typesetting.

Figures and *photographs* should be enclosed separately and protected with cardboard to avoid creasing in the mails. Only glossy prints can be used. Line drawings and graphs should be submitted on white paper or tracing cloth, and not on cross section paper. Colored illustrations can only be reproduced at the expense of the author.

References should be arranged in alphabetic order according to authorship, with the date following the author's name,* e.g.:

1. Snyder, T., and Meleney, H. 1941, The excystation of *Endamoeba histolytica* in bacteriologically sterile media, *Am. J. Trop. Med.* **21:** 63–73.

Citation in the text should be by author and date.

Proprietary names of drugs or chemicals registered by commercial pharmaceutical firms may not be used in the text if there is a generic name available in the U.S. Pharmacopoeia, New and Nonofficial Remedies of the A.M.A., or the latest editions of standard medical dictionaries. In certain cases where the generic name is relatively unknown, the registered name may follow it in parentheses in the title, at the first citation in the text, and in the conclusions.

All papers should end with a brief summary.

* This change in style of reference from that of the former American Journal of Tropical Medicine will go into effect in the next number. This issue was already in type when the decision was made.

SMITH SPRAYERS

FINEST QUALITY AND PERFORMANCE

FOR MOSQUITO AND MALARIA CONTROL
BY THE ORIGINATORS OF SPRAYERS FOR 65 YEARS

FOR RESIDUAL HOUSE SPRAYING OR LARVICIDING OPERATIONS
—— SMALLER CAPACITY SPRAYERS ARE AVAILABLE ——

SMITH D.D.T. SPRAYER

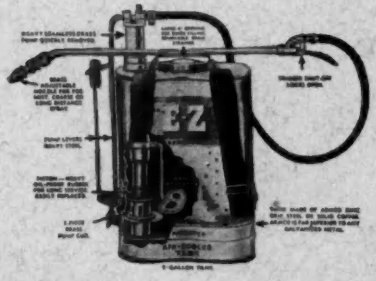
The SMITH DDT Sprayer consists of a 4 gallon heavy welded galvanized corrugated steel tank, tested for hard service; equipped with brass air pump and brass air-check valve; adjustable carrying strap with snap ends, air-pressure gauge, 7-foot length of oil resisting hose; automatic shut-off control; 2-foot extension rod and all brass precision-built adjustable or fixed type nozzles. A custom built, superior sprayer. Heavy duty industrial use.

One in case. Shipping weight—13 lbs.

SMITH No. 26-B GIANT SPRAY PUMP

A real high-class bucket spray pump with 1¼ in. diameter. Solid Brass Pump cylinder for all spraying work. Height 25 in; 3 ft. ¾ in. hose; and brass adjustable or fixed type nozzle.
A big healthy All Brass Bucket Spray Pump. Develops a very high pressure. Smooth and easy to operate.
One in case. Shipping weight—6 lbs.

SMITH E-Z KNAPSACK SPRAYER



Maintains continuous high pressure, with only easy slow pumping. Pump entirely of brass to stand long hard usage. Equipped with best grade, high-pressure hose, 2-foot brass extension pipe with adjustable brass or fixed type nozzle. New shut-off control may be locked open for continuous spray. Capacity 4 to 5 gallons. Has form-fitting ventilated tank, keeping the back dry and comfortable. Carrying handle; and dasher type agitator which keeps liquid thoroughly mixed. Brass liquid strainer.

One in case. Shipping weight—20 lbs.

D. B. SMITH & COMPANY • • UTICA 2, NEW YORK, U. S. A.
—— ESTABLISHED 1888 ——

DIFCO

Bacto

SENSITIVITY DISKS

Bacto-Sensitivity Disks are sterile paper disks containing known amounts of the more commonly employed antibiotics.

Bacto-Sensitivity Disks are used in determining the relative sensitivity of microorganisms to antibiotics.

Bacto-Sensitivity Disks are available in three concentrations of the following antibiotics:

PENICILLIN
CHLOROMYCETIN®
STREPTOMYCIN
TERRAMYCIN
DIHYDROSTREPTOMYCIN
AUREOMYCIN

•

DIFCO LABORATORIES

Detroit 1, Michigan

Leaflet No. 131 descriptive of Bacto-Sensitivity Disks available upon request